Journal of the Fisheries Research Board of Canada

Journal de l'office des recherches sur les pêcheries du Canada

Volume 35, No. 1, January 1978

Volume 35, nº 1, janvier 1978

Fisheries and Environment Canada
Fisheries and Marine Service

Pêches et Environnement Canada
Service des pêches et de la mer

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Scientific Information and Publications Branch | Direction de l'information et des publications scientifiques

Ottawa K1A 0E6

Volume 35, No. 1, January 1978 | Volume 35, nº 1, janvier 1978

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Journal of the Fisheries Research Board of Canada

Volume 35, No. 1, January 1978

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Relation of Water Temperature to Infections of Coho Salmon (*Oncorhynchus kisutch*), Chinook Salmon (*O. tshawytscha*), and Steelhead Trout (*Salmo gairdneri*) with *Aeromonas salmonicida* and *A. hydrophila*[1]

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Groberg, W. J. Jr., R. H. McCoy, K. S. Pilcher, and J. L. Fryer. 1978. Relation of water temperature to infections of coho salmon (*Oncorhynchus kisutch*), chinook salmon (*O. tshawytscha*), and steelhead trout (*Salmo gairdneri*) with *Aeromonas salmonicida* and *A. hydrophila*. J. Fish. Res. Board Can. 35: 1–7.

Juvenile steelhead trout (*Salmo gairdneri*), coho salmon (*Oncorhynchus kisutch*), and spring chinook salmon (*O. tshawytscha*) were infected by intraperitoneal or intramuscular injection with *Aeromonas salmonicida* or *A. hydrophila* at seven temperatures from 3.9 to 20.5°C. At 3.9 and 6.7°C, mortality in fish infected with *A. salmonicida* varied from 2 to 26% among the three salmonid species. At 20.5°C 93–100% of these animals died within 2 or 3 days; at 6.7°C or lower the fish survived for 12–23 days. Growth of *A. salmonicida* in vitro was influenced by temperature in a manner very similar to its influence on the in vivo infection. Comparable experiments with *A. hydrophila* gave results much like those with *A. salmonicida*, though some differences were noted. At a temperature of 20.5°C percent mortality ranged from 64 to 100%. At 9.4°C or below no deaths attributed to *A. hydrophila* occurred. Fatally infected fish died more rapidly at the higher temperatures.

Key words: Aeromonas salmonicida, A. hydrophila, water temperature, furunculosis, motile aeromonas septicemia, coho salmon, chinook salmon, steelhead trout

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Nous avons injecté *Aeromonas salmonicida* ou *A. hydrophila*, par voie intrapéritonéale ou intramusculaire, dans des jeunes truites steelhead (*Salmo gairdneri*), saumons coho (*Oncorhynchus kisutch*) et saumons chinook de printemps (*O. tshawytscha*) à sept

[1]Oregon Agricultural Experiment Station Technical Paper No. 4482.

Printed in Canada (J4740)
Imprimé au Canada (J4740)

températures comprises entre 3.9 et 20.5°C. A 3.9 et 6.7°C, la mortalité chez les poissons infectés par *A. salmonicida* varie de 2 à 26% pour les trois espèces de salmonidés. A 20.5°C, de 93–100% de ces poissons meurent en dedans de 2 ou 3 jours; à 6.7°C ou moins, les poissons survivent pendant 12–23 jours. La température exerce une influence sur la croissance de *A. salmonicida* in vitro à peu près de la même façon que dans une infection in vivo. Les résultats obtenus à la suite d'expériences comparables avec *A. hydrophila* ressemblent beaucoup à ceux obtenus avec *A. salmonicida*, avec quelques différences toutefois. A une température de 20.5°C, la mortalité varie de 64 à 100%. A 9.4°C ou moins, il ne se produit pas de mortalité attribuable à *A. hydrophila*. Les poissons fatalement infectés meurent plus rapidement aux températures plus élevées.

Received February 23, 1977
Accepted September 8, 1977

Reçu le 23 février 1977
Accepté le 8 septembre 1977

FISH furunculosis was first reported in hatchery trout from Germany by Emmerich and Weibel (1894). The causative agent was referred to as *Bacterium salmonicida* (*Aeromonas salmonicida*). Since then the disease has been found widespread throughout Europe and the United States. Numerous studies have been made of the pathogen and the disease it causes, many of which are reviewed by McGraw (1952) and Herman (1968). High water temperatures favor outbreaks of furunculosis, and Mackie et al. (1933) have reported that the temperature must be between 12.7 and 18.8°C before a serious epizootic will take place in nature.

Aeromonas hydrophila (formerly *A. liquefaciens*) is a common freshwater bacterium and is thus often present in the same water as fish (Bullock et al. 1971). It has been implicated in diseases of both warm- and cold-water fish, usually causing epizootics when the fish are under stress conditions. Motile aeromonas septicemia of pondfish and salmonids is attributed to this organism, and the disease is recognized worldwide among cultured fish.

Although it is common knowledge that higher summer water temperatures are more favorable for the development of these two bacterial diseases than the lower fall and winter temperatures, there appeared to be a need for greater precision in the definition of the favorable and unfavorable limits. To determine this, the effect of water temperature on infections of juvenile coho salmon (*Oncorhynchus kisutch*), chinook salmon (*O. tshawytscha*), and steelhead trout (*Salmo gairdneri*) by *A. salmonicida* and *A. hydrophila* was investigated.

Materials and Methods

Experiments were carried out in duplicate and in a manner similar to those reported earlier with *Flexibacter columnaris* (Holt et al. 1975). Groups of the three salmonid species containing either 25 or 35 fish were tested at seven water temperatures from 3.9 to 20.5°C in increments of 2.8°C. Each group of fish was acclimated to the desired experimental temperature as described by Holt et al. (1975).

Two strains of *A. salmonicida* were used in the experiments. Strain As-5-G was isolated from the kidney of a coho salmon during an epizootic of furunculosis at the Siletz Hatchery in Oregon. Stock cultures were preserved by cultivating the organism in the following medium: 10 g of peptone (Difco), 5 g of glucose, 10 g of beef extract, and 5 g of sodium chloride per litre of water (PBG broth). The cultures were centrifuged, resuspended in sterile skim milk, and lyophilized. The second strain, SS-70 BE-3, was isolated from the kidney of a diseased chinook salmon at the South Santiam Hatchery in Oregon. It was passed through a series of 13 transfers in juvenile coho salmon by intraperitoneal inoculation of a suspension of kidney tissue from the fish infected in the preceding transfer. Kidney tissue from the last fish in the series was macerated, suspended in skim milk, and lyophilized.

Aeromonas hydrophila strain K-1, used in experiments with spring chinook salmon and steelhead trout, was isolated from the kidney of an American shad (*Alosa sapidissima*) during an epizootic in Coos Bay, Oregon. Stock cultures were maintained on slants of the PBG medium with 1.5% agar added and overlaid with sterile mineral oil.

Aeromonas hydrophila strain JZ-45 was used in the experiments with coho salmon. It was isolated from a juvenile spring chinook salmon at the Oregon State University Fish Disease Laboratory, where it was causing fatal infections in fish held at 17.8 and 20.5°C.

Experimental infections were initiated, after the fish had been acclimated to the desired temperatures, by the intramuscular or intraperitoneal injection of 0.05 or 0.1 mL of a culture of the pathogen grown in brain heart infusion broth (BHI) (Difco) or PBG broth, suitably diluted to contain from 10 to 40 LD50/mL. Parenteral injection of *A. salmonicida* and *A. hydrophila* was the only reliable method available to produce fatal infection in a large percentage of experimental fish.

Infections were confirmed at necropsy by streaking small fragments of kidney tissue on plates of furunculosis or BHI agar. Plates were incubated at room temperature when *A. salmonicida* was the test organism and at 37°C for *A. hydrophila*. *Aeromonas salmonicida* was identified by the Gram stain (negative), lack of motility, production of brown pigment, failure to grow at 37°C, and formation of cytochrome oxidase and catalase. Agglutination by specific antiserum was used in certain experiments, but this method was not satisfactory with strain SS-70 BE-3 because of the autoagglutinating property of this organism. *Aeromonas hydrophila* was identified by the Gram stain (negative), motility, ability to grow at 37°C, and agglutination by specific antiserum.

The relationship of temperature to the growth rate of *A. salmonicida* was determined in vitro. Peptone–beef-extract–glucose (PBG) broth was inoculated with the As-5-G strain of the organism to give a concentration of 1.9×10^5 cells/mL, and distributed in 100×13 mm screw-cap tubes in 3-mL aliquots. Groups of these tubes were then incubated at each temperature used in the fish experiments. Growth was measured by determining the optical density at 650 nm at various intervals during an 80-h incubation

TABLE 1. Effect of water temperature on mortality of juvenile coho salmon (*Oncorhynchus kisutch*)[a] infected with *Aeromonas salmonicida*. NT = not tested.

Water temp (°C)	Fraction of each group that died				% mortality: 2 expt combined[c]		% infected fish yielding cultures of *A. salmonicida*[d]		Mean time from infection to death (days)
	Expt 1		Expt 2						
	Infected[b]	Controls	Infected[b]	Controls	Infected	Controls	Fatally infected	Survivors	
20.5	25/25	2/25	25/25	0/25	100	4	82	0	3.5
17.8	24/25	3/25	23/25	5/25	94	16	66	0	4.5
15.0	16/25	0/25	19/25	0/25	70	0	71	0	6.8
12.2	9/25	0/25	12/25	0/25	42	0	52	0	9.0
9.4	11/25	0/25	12/25	0/25	46	0	60	0	12.9
6.7	2/25	0/25	4/25	0/25	12	0	100	0	NT
3.9	4/25	0/25	3/25	0/25	14	0	86	NT	NT

[a]Average weight of fish when experiment started was 28 g.

[b]Fish were infected by intramuscular injection of about 2 LD50 doses (~110 organisms) of a 48-h broth culture of *A. salmonicida* strain As-5-G. Control fish received a sham injection of a sterile filtrate of the broth diluted in saline.

[c]The least significant difference between percent mortality values was determined by analysis of variance to be 14.1% at $P = 0.05$.

[d]All groups of fish were held at the indicated temperatures for 55 days. Dead fish were collected daily, necropsied, and cultures made from kidney tissue.

TABLE 2. Effect of water temperature on mortality of juvenile chinook salmon (*Oncorhynchus tshawytscha*)[a] infected with *Aeromonas salmonicida*. ND = no data obtained.

Water temp (°C)	Fraction of each group that died				% mortality: 2 expt combined[c]		% infected fish yielding cultures of *A. salmonicida*[d]		Mean time from infection to death (days)
	Expt 1		Expt 2						
	Infected[b]	Controls	Infected[b]	Controls	Infected	Controls	Fatally infected	Survivors	
20.5	22/22	2/22	20/23	0/25	93	4	100	0	2.3
17.8	18/25	2/25	19/25	3/25	74	10	100	0	3.4
15.0	13/25	3/25	15/25	2/25	56	10	100	23	5.9
12.2	16/25	5/25	19/25	3/25	70	16	100	33	6.9
9.4	12/25	2/25	12/25	1/25	48	6	96	15	9.4
6.7	4/25	4/25	9/25	0/25	26	8	100	19	12.2
3.9	2/25	0/25	2/25	3/25	8	6	0	61	ND

[a]Average weight of fish when experiment started was 19 g.

[b]Fish were infected by intraperitoneal injection of 0.1 mL of a 19-h culture of *A. salmonicida* strain SS-70 BE-3 in BHI broth, diluted to contain about 18 LD50 (~5400 organisms). Control fish received a sham injection of 0.1 mL of sterile PBS.

[c]The least significant difference between percent mortality values was determined by analysis of variance to be 13.8% at $P = 0.05$.

[d]All groups of fish were held at the indicated temperatures for 13 days. The experiment was terminated at that time due to extensive tail rot in experimental and control groups at 12.2°C and below. Dead fish were collected daily, necropsied, and cultures made from kidney tissue. After termination, all surviving experimental fish and five control fish from each group were necropsied and cultures prepared.

period. Similar measurements of growth rate in vitro were made with the K-1 strain of *A. hydrophila*. The initial concentration of cells was 10^4–10^5/mL.

Results

Effect of Temperature on *Aeromonas salmonicida* Infections

Among the groups of juvenile coho salmon infected with *A. salmonicida*, the percent mortality decreased from 100% at 20.5°C to 12 and 14% at 6.7 and 3.9°C, respectively (Table 1). Not every 2.8°C reduction in water temperature significantly influenced the percent mortality. However, the mortality was significantly lower at 15.0°C than at 17.8°C; at 12.2°C than at 15.0°C; and at 6.7°C than at 9.4°C. The data indicate clearly that the development of fatal disease in juvenile coho salmon was suppressed at water temperatures of 3.9 and 6.7°C, and that it was progressively enhanced at temperatures of 9.4°C and above.

TABLE 3. Effect of water temperature on mortality of juvenile steelhead trout (*Salmo gairdneri*)[a] infected with *Aeromonas salmonicida*.

Water temp (°C)	Fraction of each group that died				% mortality: 2 expt combined[c]		% infected fish yielding cultures of *A. salmonicida*[d]		Mean time from infection to death (days)
	Expt 1		Expt 2						
	Infected[b]	Controls	Infected[b]	Controls	Infected	Controls	Fatally infected	Survivors	
20.5	24/25	4/25	24/25	1/25	96	10	92	0	2.7
17.8	18/25	4/25	24/25	0/25	84	8	93	0	5.3
15.0	14/25	0/25	13/25	0/25	54	0	96	0	4.9
12.2	9/25	5/25	6/25	1/25	30	12	100	6	15.3
9.4	6/25	1/25	8/25	1/25	28	4	93	0	13.5
6.7	0/25	0/25	5/25	0/25	10	0	100	0	20.3
3.9	1/25	0/25	0/25	0/25	2	0	100	2	23.0

[a]Average weight of fish when experiment started was 35 g.

[b]Fish were infected by intraperitoneal injection of 0.1 mL of a 19-h culture of *A. salmonicida* strain SS-70 BE-3 in BHI broth, diluted to contain about 2 LD50 (~600 organisms). Control fish received a sham injection of 0.1 mL of sterile PBS.

[c]The least significant difference between percent mortality values was determined by analysis of variance to be 16.2% at $P = 0.05$.

[d]All groups of fish were held at the indicated temperatures for 33 days. Dead fish were collected daily, necropsied, and cultures made from kidney tissue. Surviving experimental fish and five control fish from each group were necropsied and cultures prepared.

TABLE 4. Effect of water temperature on mortality of juvenile steelhead trout (*Salmo gairdneri*)[a] infected with *Aeromonas hydrophila*. NT = not tested; ND = no data obtained since no fish died.

Water temp (°C)	Fraction of each group that died				% mortality: 2 expt combined[c]		% infected fish yielding cultures of *A. hydrophila*[d]		Mean time from infection to death (days)
	Expt 1		Expt 2						
	Infected[b]	Controls	Infected[b]	Controls	Infected	Controls	Fatally infected	Survivors	
20.5	18/35	0/35	27/35	0/35	64	0	68	28	1.7
17.8	26/35	0/35	21/35	0/35	67	0	81	17	1.8
15.0	9/35	1/35	18/35	0/35	39	1	93	7	2.1
12.2	11/35	0/35	17/35	0/35	40	0	78	2	3.3
9.4	0/35	0/35	0/35	0/35	0	0	0	0	ND
6.7	0/35	0/35	0/35	0/35	0	0	0	NT	ND
3.9	0/35	0/35	0/35	0/35	0	0	0	NT	ND

[a]Average weight of fish when experiment started was 25 g.

[b]Fish were infected by intramuscular injection of about 0.5 LD50 ($\sim 2.2 \times 10^7$ organisms) of a 24-h culture of *A. hydrophila* strain K-1 in PBG broth. Control fish received a sham injection of 0.05 mL of sterile PBG broth.

[c]The least significant difference between percent mortality values was determined by analysis of variance to be 16.1% at $P = 0.05$.

[d]All groups of fish were held at the indicated temperatures for 27 days. Dead fish were collected daily, necropsied, and cultures made from kidney tissue. Forty of the uninfected control fish were also examined by culture and one was positive for *A. hydrophila*.

Aeromonas salmonicida was recovered from a majority of the individuals in each temperature group that succumbed to the infection (Table 1). These recoveries strengthen the evidence that death was due to the aeromonas infection. The remaining fish in the groups at 15.0°C and below, from which the organism was not recovered, may also have died from the infection, since control fish, which had received a sham injection and were held under the same conditions, all remained healthy.

Data concerning mean time between infection and death (Table 1) in each temperature group were subjected to regression analysis. A linear relationship was found between water temperature (X) and the $\log_{10}$ of the mean interval (in days) between infection and death ($\hat{Y}$). The regression line is $\hat{Y} = 1.5912 - 0.05151\ X$, where X = degrees Celsius. The correlation coefficient (−0.8849) is significant. The R^2 value of 0.7832 indicates that the time to death was about 78% temperature related.

Results of similar experiments conducted with *A. salmonicida* and chinook salmon and steelhead trout

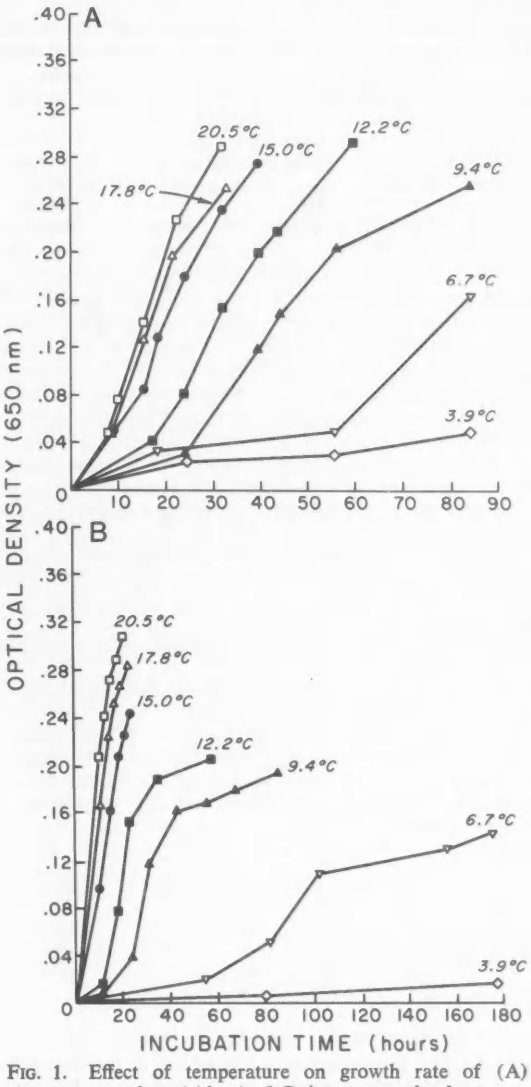

FIG. 1. Effect of temperature on growth rate of (A) *Aeromonas salmonicida* As-5-G in peptone–beef-extract–glucose broth and (B) *A. hydrophila* K-1 in peptone–beef-extract–glucose broth.

are given in Tables 2 and 3, respectively. With these salmonids, regression analysis also indicated that there was a linear relationship between water temperature and the $\log_{10}$ of the mean interval between infection and death. With chinook salmon the regression line is $\hat{Y} = 1.4164 - 0.04916\,X$. The correlation coefficient is -0.7901, a significant value, and the R^2 value of 0.6243 indicates that the time to death was about 62% temperature related. With steelhead trout, the regression line is $\hat{Y} = 1.6490 - 0.05701\,X$. The correlation coefficient was -0.7264, a significant value, and the R^2 value of 0.5276 indicates that the time to death was about 53% temperature related.

When the growth rate of *A. salmonicida* was measured in vitro at the experimental temperatures we noted that the organism grew very slowly at 3.9 and 6.7°C, and the rate of growth increased progressively with each 2.8°C increase in temperature, reaching a maximum at 20.5°C (Fig. 1A). Thus the effect of temperature on the growth rate of the bacterium appears to follow a pattern similar to its effect on mortality among infected fish. Temperature may also influence the host defense mechanisms; it certainly affects the growth rate of the pathogen, which in turn has a major influence on mortality.

EFFECT OF TEMPERATURE ON *Aeromonas hydrophila* INFECTIONS

In the experiments with juvenile steelhead trout infected with *A. hydrophila*, mortality was approximately the same at 20.5 and 17.8°C (Table 4). Mortality was significantly reduced at 15.0°C and at 12.2°C, where 39 and 40% of the fish died, respectively. The progress of infection was apparently retarded or halted at 9.4, 6.7, and 3.9°C, as no deaths occurred at these temperatures. Thus temperatures from 12.2 to 20.5°C were favorable for the development of this infection in steelhead trout, and increased mortality was correlated with increased temperature. However, this relationship was less striking than in the *A. salmonicida* infections because no deaths occurred at 9.4°C or lower.

Among the infected fish that died, 68–93% in the various temperature groups yielded cultures of *A. hydrophila* on necropsy (Table 4). These data provide supporting evidence that death of these fish was caused by infection with this organism. The remaining fish in these groups, from which the organism was not recovered, may also have died from the infection, since all but one of the control fish at these temperatures, which had received a sham injection, remained healthy. Among the surviving but infected groups of fish, some still harbored the organism in the kidney. Because of the small number of survivors from which the organism was recovered, the differences in the percentages of positive cultures between any two temperature levels differing by a 2.8°C increment are not statistically significant. However, the 28% value at 20.5°C differs significantly from the 7, 2, and 0% values at 15.0, 12.2, and 9.4°C, respectively. Also, the 17% value at 17.8°C differs significantly from the 2% value at 12.2°C. Among 40 control fish from the four highest temperature groups examined by culture, 39 were negative and *A. hydrophila* was isolated from one. These data suggest that within the temperature range studied, the higher temperatures favor proliferation of the pathogen, while at lower temperatures the microorganisms were not able to survive in the tissues of the host.

Results of experiments conducted with *A. hydrophila* and chinook and coho salmon are given in Tables 5

TABLE 5. Effect of water temperature on mortality of juvenile spring chinook salmon (*Oncorhynchus tshawytscha*)[a] infected with *Aeromonas hydrophila*. ND = no data obtained since no fish died; . . ., mean time to death not recorded because deaths not due to *A. hydrophila*.

Water temp (°C)	Fraction of each group that died				% mortality: 2 expt combined[c]		% infected fish yielding cultures of *A. hydrophila*[d]		Mean time from infection to death (days)
	Expt 1		Expt 2						
	Infected[b]	Controls	Infected[b]	Controls	Infected	Controls	Fatally infected	Survivors	
20.5	16/25	1/25	18/25	0/25	68	2	97	0	1.2
17.8	19/25	2/25	12/25	0/25	62	4	94	0	1.4
15.0	14/25	0/25	14/25	1/25	56	2	97	0	1.3
12.2	7/25	0/25	12/25	1/25	38	2	100	0	3.1
9.4	0/25	2/25	0/25	0/25	0	4	0	0	ND
6.7	3/25	0/25	4/25	6/25	14	12	0	0	. . .
3.9	4/25	5/25	2/25	2/25	12	14	0	0	. . .

[a]Average weight of fish when experiment started was 18 g.

[b]Fish were infected by intraperitoneal injection of 0.05 mL of an 18-h culture of *A. hydrophila* strain K-1 in BHI broth, diluted in PBS to contain about 2 LD50 ($\sim 1.2 \times 10^7$ organisms). Control fish received a sham injection of 0.05 mL of sterile PBS.

[c]The least significant difference between percent mortality values was determined by analysis of variance to be 13.3% at $P = 0.05$.

[d]All groups of fish were held at the indicated temperatures for 12 days. Dead fish were collected daily, necropsied, and cultures made from kidney tissue. After termination, all surviving experimental fish and five control fish from each group were necropsied and cultures prepared.

TABLE 6. Effect of water temperature on mortality of juvenile coho salmon (*Oncorhynchus kisutch*)[a] infected with *Aeromonas hydrophila*. NT = not tested; ND = no data obtained.

Water temp (°C)	Fraction of each group that died				% mortality: 2 expt combined[c]		% infected fish yielding cultures of *A. hydrophila*[d]	Mean time from infection to death (days)
	Expt 1		Expt 2					
	Infected[b]	Controls	Infected[b]	Controls	Infected	Controls	Fatally infected	
20.5	35/35	2/35	36/36	0/35	100	3	100	1.0
17.8	36/36	1/35	34/34	0/35	100	1	100	1.5
15.0	33/35	0/35	35/35	0/35	97	0	100	2.3
12.2	16/35	0/35	13/35	0/35	41	0	100	4.7
9.4	2/35	0/35	0/35	0/35	3	0	NT	ND
6.7	0/35	0/35	0/35	0/35	0	0	NT	ND[e]
3.9	0/35	0/35	0/35	0/35	0	0	NT	ND[e]

[a]Average weight of fish when experiment started was 18 g.

[b]Fish were infected by intramuscular injection of 0.05 mL of an 18-h culture of *A. hydrophila* strain JZ-45 in BHI broth, diluted to contain about 1.5 LD50 ($\sim 2.9 \times 10^7$ cells). Control fish received a sham injection of 0.05 mL of sterile BHI broth.

[c]The least significant difference between percent mortality values was determined by analysis of variance to be 5.02% at $P = 0.05$.

[d]All groups of fish were held at the indicated temperatures for 25 days. Dead fish were collected daily, necropsied, and cultures made from kidney tissue. *Aeromonas hydrophila* was recovered from 3 of 40 uninoculated control fish examined at the end of the experiment.

[e]No data obtained since no fish died.

and 6, respectively. As with steelhead trout, fatal infections in chinook and coho salmon were progressively enhanced as the temperature was increased from 12.2°C and were virtually all suppressed at temperatures of 9.4°C and below. The *A. hydrophila* experimental data were not subjected to regression analysis because a significant linear relationship did not appear to exist between water temperature and the logarithm of the mean interval between infection and death.

Measurement of the growth rate of *A. hydrophila* in vitro at the experimental temperatures showed that 3.9°C growth was barely measurable and was quite slow at 6.7°C (Fig. 1B). The rate increased significantly at 9.4°C and continued to increase with further increases in temperature until a maximum was reached at 20.5°C (Fig. 1B). Temperatures of 15.0–20.5°C, which gave the highest in vitro growth rates, are also those that produced the highest mortality and the shortest

mean times from infection to death in inoculated fish. The three lowest temperatures, (3.9, 6.7, 9.4°C) reduced growth rates and consistently suppressed development of fatal infections.

Discussion

These studies clearly demonstrate that higher temperatures accelerated the progress of infection by *A. salmonicida* and *A. hydrophila*, while lower temperatures retarded it. Results obtained with *A. salmonicida* do not conflict with an earlier report indicating that water temperatures must be 12.7°C or above before a serious epizootic of furunculosis will occur (Mackie et al. 1933). *Aeromonas hydrophila* epizootics are precipitated by stress conditions (Bullock et al. 1971) and infections in salmonids are usually associated with elevated water temperatures. Our data confirm the severity of motile aeromonas septicema at increased temperatures. In addition, these studies provide a more precise examination of the effects of temperature on mortality due to aeromonas infections than was previously available.

Water temperatures in many rivers of the Pacific Northwest from May through October are in a range favorable for the progress of aeromonad infections of salmonids. During this period threshold water temperatures for these diseases are reached and maxima of 21°C are not uncommon. Our data indicate that it would be undesirable to allow additional sources of heat to enter these rivers, since added heat during this period could only serve to enhance the severity of both diseases. Lower water temperatures favorable to the host generally occur from November through April. Increasing water temperatures would shorten this interval and extend the period when conditions are favorable for the pathogen.

Acknowledgments

The Oregon Department of Fish and Wildlife generously provided the large numbers of juvenile salmon and trout required for the experiments.

We thank Mr J. L. Zinn for his technical assistance. This project was financed by the Environmental Protection Agency under grant numbers 18050 DIJ and R 800171.

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Relation of Water Temperature to Bacterial Kidney Disease in Coho Salmon (*Oncorhynchus kisutch*), Sockeye Salmon (*O. nerka*), and Steelhead Trout (*Salmo gairdneri*)[1]

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SANDERS, J. E., K. S. PILCHER, AND J. L. FRYER. 1978. Relation of water temperature to bacterial kidney disease in coho salmon (*Oncorhynchus kisutch*), sockeye salmon (*O. nerka*), and steelhead trout (*Salmo gairdneri*). J. Fish Res. Board Can. 35: 8–11.

Juvenile coho salmon (*Oncorhynchus kisutch*), sockeye salmon (*O. nerka*), and steelhead trout (*Salmo gairdneri*) were infected by intraperitoneal injection of a suspension of the causative organism of bacterial kidney disease at seven experimental temperatures from 3.9 to 20.5°C. In coho salmon and steelhead trout percent mortality was maximal (78–100%) in the range of 6.7–12.2°C. As the temperature increased above 12.2°C mortality declined progressively; at 20.5°C it was only 8–14%. The two highest temperatures had some suppressing effect on the disease, which was greatest at 20.5°C. In sockeye salmon this was not apparent, as percent mortality was essentially 100% at all temperatures from 6.7 to 20.5°C; this species appeared to be highly susceptible to the disease agent. Temperature also influenced the mean number of days between infection and death. This interval was shortest in all three species of salmonids at the higher temperatures, varying from 21 to 34 days at 15.0–20.5°C. It increased progressively as temperature decreased below this range; at 6.7°C it varied from 60 to 71 days among the three species.

Key words: bacterial kidney disease, temperature, salmonids, *Corynebacterium*, coho salmon, sockeye salmon, steelhead trout

SANDERS, J. E., K. S. PILCHER, AND J. L. FRYER. 1978. Relation of water temperature to bacterial kidney disease in coho salmon (*Oncorhynchus kisutch*), sockeye salmon (*O. nerka*), and steelhead trout (*Salmo gairdneri*). J. Fish Res. Board Can. 35: 8–11.

Nous avons injecté, par voie intrapéritonéale, une suspension de l'organisme responsable de la maladie bactérienne du rein dans des jeunes saumons coho (*Oncorhynchus kisutch*), saumons nerka (*O. nerka*), et truites steelhead (*Salmo gairdneri*) à sept températures expérimentales comprises entre 3.9 et 20.5°C. Chez les saumons coho et les truites steelhead, la mortalité est maximale (78–100%) entre 6.7–12.2°C. La mortalité diminue progressivement à mesure que la température augmente une fois cet intervalle dépassé; à 20.5°C, elle n'est que de 8–14%. Les deux températures les plus élevées empêchent quelque peu la maladie de se manifester, surtout à 20.5°C. Ceci n'est pas apparent chez le saumon nerka, la mortalité étant essentiellement de 100% à toutes les températures comprises entre 6.7 et 20.5°C; cette espèce semble très susceptible à l'agent pathogène. La température affecte également le nombre moyen de jours qui séparent l'infection de la mort. Le plus court intervalle se rencontre chez toutes les trois espèces de salmonidés aux plus hautes températures, allant de 21 à 34 jours entre 15.0–20.5°C. L'intervalle augmente progressivement à mesure que la température s'abaisse au-dessous de cette gamme; à 6.7°C, elle varie de 60 à 71 jours chez les trois espèces.

Received February 23, 1977
Accepted September 8, 1977

Reçu le 23 février 1977
Accepté le 8 septembre 1977

BACTERIAL kidney disease is an infection of salmonid fish caused by a gram-positive diplobacillus classified as an unnamed species of *Corynebacterium*. The organism produces a chronic bacteremia, with foci of infection occurring in various visceral organs, most commonly the kidney. The bacterium is fastidious in its growth requirements, and on a medium containing cysteine and blood, about 1 wk is required for visible growth at 18°C (Ordal and Earp 1956; Smith 1964). Early work on this disease was reviewed by Rucker et al. (1954) and Bell (1961).

Some outbreaks of the disease occur in spring and others in the fall when water temperature is 10°C or

[1]Oregon Agricultural Experiment Station Technical Paper No. 4481.

Printed in Canada (J4741)
Imprimé au Canada (J4741)

TABLE 1. Effect of water temperature on mortality of juvenile coho salmon (*Oncorhynchus kisutch*) experimentally infected with the causative agent of bacterial kidney disease (strain HR-4-67).

Water temp (°C)	Fraction of each group that died[a]				% mortality: 2 expt combined[b,c]		% deaths due to bacterial kidney disease		Mean time from infection to death (days)
	Expt 1		Expt 2						
	Infected	Controls	Infected	Controls	Infected	Controls	Infected	Controls	
20.5	5/21	1/20	5/23	2/23	14	0	60	0	23
17.8	10/22	0/23	12/22	2/22	41	0	82	0	24
15.0	23/25	0/25	15/25	0/25	76	0	100	0	34
12.2	21/21	0/25	21/21	0/25	100	0	100	0	37
9.4	25/25	3/23	22/23	0/25	98	2[d]	100	33[d]	51
6.7	21/21	0/25	20/23	1/25	93	2[d]	100	100[d]	71
3.9	11/22	0/23	15/19	1/24	63	0	100	0	81

[a]All groups of fish were held at the indicated temperature for 112 days.
[b]Only dead fish in which the specific pathogen was demonstrated at necropsy were considered in calculating percent mortality.
[c]The least significant difference between percent mortality values was determined by analysis of variance to be 20.18% at $P = 0.05$.
[d]These control fish were found to be infected with the causative agent of bacterial kidney disease.

below. In water of 13–18°C the course of the disease may be accelerated and mortalities heavy (Bullock et al. 1971). However, in brook trout (*Salvelinus fontinalis*) greater losses were observed at 7°C than at 12.5°C (Wolf and Dunbar 1959). The objective of our experiments is to obtain more precise data concerning the effect of water temperature on kidney disease in coho salmon (*Oncorhynchus kisutch*), sockeye salmon (*O. nerka*), and steelhead trout (*Salmo gairdneri*).

Materials and Methods

The design of the experiments and acclimation procedures were almost identical with those used in studies with *Flexibacter columnaris* (Holt et al. 1975). However, seven water temperatures varying from 3.9 to 20.5°C were employed instead of eight, and the studies were conducted in duplicate.

All fishes selected for this study had no history of infection with bacterial kidney disease, although detection methods are of limited effectiveness in determining light infections. Bacterial kidney disease was subsequently diagnosed in dead fish from the hatchery-production group from which the coho salmon used in this experiment were obtained.

Two strains of the *Corynebacterium* species causing kidney disease were used in the experiments. Strain HR-4-67, isolated from a chinook salmon at the Hood River Hatchery in 1967, was used in the experiments with coho salmon. Experiments with sockeye salmon and steelhead trout were carried out with strain RB-1-73, isolated from a chinook salmon at the Round Butte Hatchery in 1973.

Stock cultures of the bacterium were maintained on an enriched medium containing cysteine (Ordal and Earp 1956) modified by substituting calf serum for human blood (CSA). Inoculum for infecting fish was prepared as a suspension of the organism in physiological saline (PBS). In experiments with coho salmon the suspension contained about 2.5×10^8 bacteria in 0.05 mL, and the fish, which averaged 6.5 g in weight, were infected by intraperitoneal injection of this volume. Steelhead trout, averaging 18 g in weight, received 2.9×10^8 bacteria by the same route, while sockeye salmon, with an average weight of 38 g, were injected with 3.3×10^8 organisms. All control fish received an equal volume of PBS.

Dead fish were collected daily during the experimental period, necropsied, and smears of kidney tissue prepared on microscope slides. The smears were gram stained and examined for the presence of the typical kidney disease bacterium. This criterion for establishing infection has limited sensitivity, so that some lightly infected animals may not be detected. In the coho salmon experiments, a second method of diagnosis was used in which kidney tissue was cultured on CSA. This method did not prove to be more sensitive than the direct microscopic procedure for detection of the organism in experimentally infected fish.

Results

In Table 1 the combined data from the two parallel experiments with coho salmon infected with the HR-4-67 strain of the kidney disease bacterium show that mortality approached 100% in the temperature range 6.7–12.2°C. As the temperature increased above 12.2°C mortality declined progressively and was only 41% at 17.8°C and 14% at 20.5°C. When the experiment ended after 112 days, mortality at 3.9°C had reached 63%, and fish were still dying from kidney disease. At this time the 15 surviving fish in the infected groups and the 46 control animals at this temperature were acclimated to 12.2°C water by the method of Holt et al. (1975). Within 2 wk all the fish in the infected groups had succumbed to kidney disease, whereas none of the control fish had died. This indicates that the pathogen was still viable in the inoculated fish held at 3.9°C and that progress of the infection was accelerated by increasing the temperature to 12.2°C. Presumably, if these fish had been allowed to remain at 3.9°C for a longer period, the mortality from kidney disease would have approached 100%.

Although 20.5 and 17.8°C were the most unfavor-

TABLE 2. Effect of water temperature on mortality of juvenile steelhead trout (*Salmo gairdneri*) experimentally infected with the causative agent of bacterial kidney disease (strain RB-1-73).

Water temp (°C)	Fraction of each group that died[a]				% mortality: 2 expt combined[b,c]		% deaths due to bacterial kidney disease		Mean time from infection to death (days)
	Expt 1		Expt 2						
	Infected	Controls	Infected	Controls	Infected	Controls	Infected	Controls	
20.5	20/24	25/26	22/24	3/23	8	0	10	0	—[d]
17.8	25/27	6/26	26/27	7/23	43	0	45	0	26
15.0	17/26	3/27	20/27	1/26	49	0	70	0	21
12.2	23/26	5/27	25/28	1/27	78	0	88	0	35
9.4	25/25	6/26	27/27	2/27	90	0	90	0	41
6.7	26/26	1/26	25/25	0/25	98	0	98	0	62
3.9	4/19	13/25	14/25	5/25	36	0	89	0	88

[a]All groups of fish were held at the indicated temperatures for 112 days.
[b]Only dead fish in which the specific pathogen was demonstrated at necropsy were considered in calculating percent mortality.
[c]The least significant difference between percent mortality values was determined by analysis of variance to be 15.33% at $P = 0.05$.
[d]At 20.5°C the number of deaths was too small to permit a reliable estimation of the mean time to death.

TABLE 3. Effect of water temperature on mortality of juvenile sockeye salmon (*Oncorhynchus nerka*) experimentally infected with the causative agent of bacterial kidney disease (strain RB-1-73).

Water temp (°C)	Fraction of each group that died[a]				% mortality: 2 expt combined[b,c]		% deaths due to bacterial kidney disease		Mean time from infection to death (days)
	Expt 1		Expt 2						
	Infected	Controls	Infected	Controls	Infected	Controls	Infected	Controls	
20.5	25/26	0/25	21/23	0/25	94	0	100	0	26
17.8	24/24	3/27	24/24	0/26	100	4[d]	100	66	23
15.0	28/28	0/24	24/24	2/26	100	4[d]	100	100	25
12.2	27/27	4/24	27/27	1/25	100	6[d]	100	60	30
9.4	24/24	0/24	26/26	0/26	100	0	100	0	43
6.7	28/28	0/27	27/27	0/27	100	0	100	0	60
3.9	14/26	0/25	14/26	0/27	54	0	100	0	70

[a]All groups of fish were held at the indicated temperatures for 90 days.
[b]Only dead fish in which the specific pathogen was demonstrated at necropsy were considered in calculating percent mortality.
[c]The least significant difference between percent mortality values was determined by analysis of variance to be 15.99% at $P = 0.05$.
[d]These control fish were found to be infected with the causative agent of bacterial kidney disease.

able temperatures for the production of fatal disease, as shown by the percent mortality values, fish that did develop fatal infection exhibited the shortest survival time, i.e. 23 and 24 days, respectively (Table 1). The mean time from infection to death increased progressively from 23 days at 20.5°C to 81 days at 3.9°C. This data was subjected to regression analysis, and the relationship between the natural log of the mean time from infection to death in days ($\hat{Y}$) and the water temperature in degrees Celsius (X) was found to be linear: $\hat{Y} = 4.7653 - 0.0864\ X$. The correlation coefficient, −0.7496, is significant at the $P = 0.05$ level; the R^2 value, 0.5619, indicates that the time to death was about 56% temperature related. Obviously the rate of progress of the disease in the fish was retarded by decreasing water temperatures.

In juvenile steelhead trout the percent mortality was greatest (78–98%) in the range 6.7–12.2°C (Table 2). When the experiment ended after 112 days, 21 of the 26 surviving fish at 3.9°C were found by microscopic examination to be harboring the kidney disease bacterium. Presumably, if they had been allowed to remain at 3.9°C for a longer period, mortality from kidney disease would have approached 100%.

Thus the data in Table 2 indicate that in the steelhead trout as in the coho salmon, the range of 6.7–12.2°C was optimal for the development of fatal infections. Higher temperatures had a suppressing effect, which was greatest at 20.5°C. A complicating factor in the steelhead trout experiment was the prevalence of tail rot in the fish population. It is presumed to be the cause of the deaths among uninoculated control groups shown

in Table 2. However, in calculating percent mortality values due to kidney disease, only those dead fish were included that harbored the specific pathogen. Thus the possible distortion of the data by nonspecific infection should be largely eliminated.

Although percent mortality was greatest in steelhead trout between 6.7 and 12.2°C, the shortest mean time to death was observed at 15 and 17.8°C (Table 2). At these higher temperatures about 50% of the steelhead trout developed fatal infections that progressed more rapidly (21 and 26 days, respectively) than in fish maintained at lower temperatures. As with coho salmon, there was a progressive increase in the mean time to death as the temperature decreased from 15 to 3.9°C.

The relationship between temperature (X) and mean time to death ($\hat{Y}$) was again linear: $\hat{Y} = 4.8001 - 0.1049\ X$. The correlation coefficient, −0.7927, is significant at the $P = 0.05$ level; R^2, 0.6284, indicates that time to death is about 63% temperature related.

The p rcent mortalities of sockeye salmon (Table 3) are obviously much different from those found for the other two salmonids. With this species mortality was essentially 100% at all water temperatures except 3.9°C. However, deaths due to bacterial kidney disease were still occurring at this temperature when the experiment ended at 90 days. If these tests had extended over a longer period, mortality might well have approached 100%. No significant suppressing effect was observed at 17.8 or 20.5°C. This result suggests that sockeye salmon were more susceptible to this pathogen than coho salmon and steelhead trout.

Seven uninoculated control fish, scattered among three different temperature groups, were found to be infected with the kidney disease organism (Table 3). These infections, like those found in the coho salmon, were probably present when the fish were obtained; however, the incidence was low, and not sufficient to invalidate the overall result.

The mean time from infection until death was shortest at the three highest temperatures, and not greatly different from the values obtained for coho salmon and steelhead trout. As water temperature decreased from 17.8 to 3.9°C the mean time to death increased progressively from 23 to 70 days. Regression analysis again revealed a linear relationship between these variables: $\hat{Y} = 4.4293 - 0.0693\ X$. The coefficient of correlation, −0.7816, is significant at the $P = 0.05$ level; R^2, 0.6109, indicates that time to death is about 61% temperature related.

Discussion

Epizootics of bacterial kidney disease have been reported to occur at temperatures ranging from 18°C (Bullock et al. 1971) to 7.2°C (Wood 1974). Wood also indicated that depending on water temperature and the intensity of initial exposure, losses from bacterial kidney diseases will occur 30–35 days after exposure at temperatures above 11.1°C and 60–90 days thereafter at temperatures of 7.2–10°C. Data obtained in our study are in general agreement with these reported observations of natural epizootics, further indicating that bacterial kidney disease is a slowly developing infection, causing death of the host over a wide temperature range.

Bacterial kidney disease is the only infection studied in this laboratory in which temperatures of 17.8 and 20.5°C exerted a suppressive effect on the disease process (Tables 1 and 2). With *Flexibacter columnaris* infection and those due to *Aeromonas salmonicida* and *A. hydrophila*, fish held at these higher temperatures experienced the greatest mortality (Holt et al. 1975; Groberg et al. 1978). In contrast, at 3.9 and 6.7°C these fish pathogens caused few deaths compared to the high mortality resulting from bacterial kidney disease.

Acknowledgments

The Oregon Department of Fish and Wildlife generously provided the large numbers of juvenile salmon and steelhead trout required for the experiments.

We thank Mr J. L. Zinn for his technical assistance. This project was financed by the Environmental Protection Agency under grant numbers 18050 DIJ and R 800171.

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Feeding Interrelations Between Age-Groups of Pumpkinseed (*Lepomis gibbosus*) and Comparisons with Bluegill (*L. macrochirus*)

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KEAST, A. 1978. Feeding interrelations between age-groups of pumpkinseed (*Lepomis gibbosus*) and comparisons with bluegill (*L. macrochirus*). J. Fish. Res. Board Can. 35: 12–27.

Analysis of the stomachs of 2435 pumpkinseeds (*Lepomis gibbosus*) showed that the diet was made up largely of chironomid larvae, molluscs, isopods, with lesser quantities of amphipods, Trichoptera larvae, and Ephemeroptera nymphs. Cladocera were only important to the very young fish. Age-class separation, in terms of taxonomic and prey-size categories, was minor, although the diet changed somewhat with age. Quantification of the diet overlaps between year-classes (by Levins index) revealed overlap levels that were moderate to high, varying seasonally between 0.5 and 1.1. They were greatest in July when the diversity of available prey organisms fell and October when several size-classes simultaneously consumed isopods, then at a peak in biomass. The feeding and spatial ecology of the pumpkinseed was compared with that of a closely related congener, the bluegill (*L. macrochirus*). The two species and their equivalent year-classes separated out partially on diet (the bluegill consuming a greater proportion of Cladocera and Trichoptera larvae; the pumpkinseed, molluscs and isopods), and partly on habitat and the height at which they foraged in the water column. Ecological overlap values between equivalent year-classes of the two species were moderate, 0.52–0.57 (Levins scale), on the basis of diet alone as well as when the habitat component was incorporated. It is suggested, however, that other differences serve to reduce these levels.

Key words: feeding, year-class diets, sunfish, intraspecific competition, interspecific competition, prey invertebrates, Centrarchidae

KEAST, A. 1978. Feeding interrelations between age-groups of pumpkinseed (*Lepomis gibbosus*) and comparisons with bluegill (*L. macrochirus*). J. Fish. Res. Board Can. 35: 12–27.

L'analyse du contenu stomacal de 2 435 crapets-soleils (*Lepomis gibbosus*) démontre que le régime alimentaire est constitué en grande partie de larves de chironomidés, de mollusques, d'isopodes et, à un degré moindre, d'amphipodes, de larves de Trichoptera et de nymphes d'Ephemeroptera. Les Cladocera n'ont d'importance que pour les très jeunes poissons. Quoique le régime change quelque peu avec l'âge, les différences entre les classes d'âge, tant dans les catégories taxonomiques que dans la taille des proies, sont mineures. Une quantification des chevauchements dans le régime d'une classe d'âge à l'autre (suivant l'indice de Levins) démontre que le degré de chevauchement varie de modéré à élevé, selon la saison, soit de 0.5 à 1.1. Le chevauchement est plus marqué en juillet, alors que la diversité des proies disponibles diminue, et en octobre, au moment où plusieurs classes d'âge consomment simultanément des isopodes qui atteignent alors un pic de biomasse. L'auteur compare l'alimentation et l'écologie spatiale du crapet-soleil avec celle d'un congénère étroitement apparenté, le crapet arlequin (*L. macrochirus*). Les classes d'âge correspondantes des deux espèces se séparent partiellement sous le rapport du régime alimentaire (le crapet arlequin consommant une plus grande proportion de larves de Cladocera et de Trichoptera, le crapet-soleil plus de mollusques et d'isopodes) et partiellement sous le rapport de l'habitat et de la hauteur à laquelle ils se nourrissent dans la colonne d'eau. Le chevauchement écologique entre classes d'âge correspondantes des deux espèces est modéré, soit de 0.52–0.57 (échelle de Levins), que l'on considère le régime seul ou que l'on y incorpore la composante habitat. On suggère cependant qu'une réduction de ces valeurs puisse être causée par d'autres différences.

Received July 5, 1976
Accepted September 2, 1977

Reçu le 5 juillet 1976
Accepté le 2 septembre 1977

Printed in Canada (J4442)
Imprimé au Canada (J4442)

STUDIES on the feeding ecology of fishes have rarely emphasized seasonal aspects and the diets of individual age- (size-) groups within species. Again few workers have endeavored to explain feeding shifts by the simultaneous study of fluctuations in the resource base. Yet it is important to try to understand the basic needs of a species and how these are obtained from the environment. An interesting facet of poikilotherms at cold temperature latitudes is the simultaneous presence in the population of several distinct age-groups. It would be advantageous to a species if these could be channeled towards different resources or habitats, thus lessening the likelihood of intraspecific competition. Yet such divergence might complicate interrelations with other species. Successful integration into an ecological system then must also involve a compromise between these two factors.

The objectives of the present study are twofold. First it investigates these aspects of feeding in a common and successful species, the pumpkinseed (*Lepomis gibbosus*). Second it applies the findings to a comparative analysis of the ecological interrelationships of the pumpkinseed with its closely related congener, the bluegill (*L. macrochirus*), comparing the diets, feeding habits, and habitat relationships of the various year-classes of the two. An attempt is made to quantify interrelationship by developing sets of ecological overlap values.

Materials and Methods

The work was carried out on the Lake Opinicon (Leeds County, Ont.) population of the two species. The study site was Cow Island Bay and the adjacent 400-m-long section of shoreline extending east towards the Queen's University Field Station. This inshore strip of sand–mud bottom is lightly to moderately weeded but has heavily weeded *Potamogeton pusillus–P. pectinatus* sections at the head of the bay. The area supports large populations of the two species and a full range of age-classes of both. The work was carried out in the ice-free months (May–November) of 1971, 1972, and 1973. There is virtually no winter feeding in these sunfishes (Keast 1968).

The fish were netted at monthly intervals over 12-day periods from the 15 to the 26 of each month. The time spread was to give as accurate a picture as possible of the prey organisms taken during the particular month. All collections were made between 1700–1900 and 0700–0900 to broadly match the major daily feeding periods of the two species (Keast and Welsh 1968), and were supplemented by night collections.

The fish caught were allocated to year-classes on the basis of studies carried out in September–October (i.e. after completion of the summer's growth) in 1968 and 1969. This entailed measuring 3200 pumpkinseeds and 4500 bluegills and defining year-class limits by the reading of the scales of 350 of each. Year-class size distributions vary little from year to year in Lake Opinicon (Keast unpublished data). Regier (1962) provides an assessment of the scale method of aging in *Lepomis*. Size-distribution data for the individual summer months (Keast unpublished data) provided the basis for the allocation of fish to year-classes.

A major problem in any study such as this is that the removal of fish from the system may, by reducing the population, disrupt normal relationships with other species and with the prey organisms. Although both species studied here have relatively high populations and can quickly recolonize areas from which fish have been removed, the numbers of fish of each age-class taken was limited to about 20/mo. The program was accordingly extended over three summers.

Diets were determined by stomach content analysis. Most prey organisms were sorted down to order and family, and for amphipods, Cladocera, molluscs, and odonate nymphs, down to genus. Counts and total weight were obtained for each group, and body lengths and widths were measured for each organism. Mollusca and Trichoptera larvae were weighed less the shell or tube.

To obtain data on seasonal changes in numbers and weights of the major benthic and planktonic prey organisms, invertebrate studies were carried out in the study site in the summer of 1973. The benthic sampling entailed removing the top couple centimetres of 0.37-m-wide strips of substrate by means of a heavy push net with metal cutting edge. The work was preceded by the laying of a gridwork of stakes set 2 m apart. Thus, individual samples were 2×0.374 m or 0.75 m^2. Series of 40 samples were taken in late afternoon and early morning (at the same time the fish were collected), between the 12 and 16, and 24 and 28 of each month (Keast 1977b). The two sets of data were grouped to obtain an average figure for each month.

Processing of the benthic samples entailed washing the material through screens. Graphs were developed on the basis of medians and interquartile ranges (which are appropriate for patchily distributed organisms). For associated data on population cycles in the zooplankton see Gibson (1974).

The justification for using benthic invertebrate data obtained in one year to interpret grouped data collected over several years is discussed in Keast (1977b). It is appropriate because of the high degree of year-to-year predictability in timing of all the major invertebrate events, such as postwinter inshore numerical buildups, emergence times (representing the loss of a resource from the system), time of appearance of new generations, and temporal and successional sequence of the population peaks in the major prey organisms.

Habitat data for the two *Lepomis* species in Lake Opinicon was developed in independent studies (Keast and Harker 1977; Keast et al. 1977) in which fish distributions and numbers at different depths and habitat types were determined by the strip count method, using face mask and snorkel, and SCUBA. This work was supplemented by selective netting of the different habitat types.

Diet diversity and niche overlaps were calculated by the formula of Levins (1968, p. 43 and 51), as follows:

$$\beta_i \text{ (niche breadth)} = 1 \Big/ \sum_{h=1}^{s} (p_{ih}^2)$$

$$\alpha_{ji} \text{ (niche overlap)} = \sum_{h=1}^{s} p_{ih} p_{jh} \Big/ \sum_{h=1}^{s} p_{ih}^2$$

where α_{ji} is the overlap of species j on species i relative to the niche breadth of species i for all h sample types of resource s; p_{ih} is the proportion of a particular item h in the diet of species i; p_{jh} is the proportion of a particular item h in the diet of species j; and p_i is the breadth of

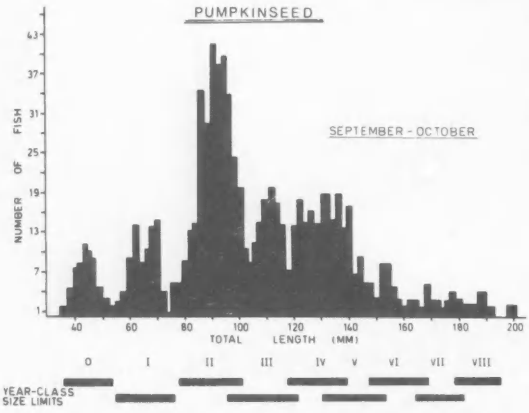

FIG. 1. Size distribution and age-class limits in September–October, based on the measurement of 3200 individuals and scale aging of 350 pumpkinseeds, Cow Island Bay, Lake Opinicon. Note that the younger year-classes are underrepresented, as they inhabit dense weed beds that are difficult to net quantitatively.

utilization of a particular resource by species *i*, in this case food. May (1975) shows that of all the niche calculations available, the Levins formulae, which exhibit good mathematical reasoning and biological relevence, seem to be the most appropriate.

Results

The size (age) structure of the Lake Opinicon pumpkinseed population at the end of the growth season is shown in Fig. 1. Year-class total body length limits at this time are as follows: Year 0, 35–53 mm; I, 55–73; II, 78–101; III, 96–120; IV, 117–138; V, 132–153; and VI, 147–168 mm.

DIETS OF THE YEAR-CLASSES OF PUMPKINSEEDS

To test whether it is justifiable to lump food data obtained at the study site in 3 succeeding yr, the diets of three age-groups of pumpkinseeds for the years 1971, 1972, and 1973 are compared for May and August in Table 1. There proved to be good general agreement. The diets are, in fact, more similar than those from different habitats at any one time (Harker 1976). Accordingly, the 3 yr of data are grouped in Table 2, which shows monthly changes in diet, and all subsequent treatments.

To broadly define year-class differences in diet, data from Table 2 are combined to give mean annual figures for the eight most important taxonomic and nine most important prey-size categories of organisms (Fig. 2 and 3). There is a basic similarity in the diets of all six year-class groups, although there are changes with age. Thus, Cladocera account for a significant number and volume of the prey organisms only in Year 0 fish, and gastropods and plant material are prominent only in the

TABLE 1. Major food organisms in terms of percent volume eaten by the Year-classes I, II, and VI–VII of *Lepomis gibbosus* in May and August in 3 successive yr. Cow Island Bay and adjacent shoreline, Lake Opinicon. Each series is based on samples of 20–25 fish. Commoner food items only. N = nymph; P = pupae; L = larvae.

	May			August		
Food organisms	1971	1972	1973	1971	1972	1973
			Year-class I			
Cladocera	7	11	5	1	2	1
Amphipoda	4	5	2	11	15	10
Isopoda	34	27	42	14	9	11
Ephemeroptera N.	6	7	8	21	17	25
Trichoptera	2	1	6			
Chironomid L.	38	42	29	44	46	51
Gastropoda	4	3	3	3	4	
			Year-class II			
Amphipoda	1		1	13	3	5
Isopoda	29	28	39	10	12	8
Ephemeroptera N.	6	8	8	8	7	7
Anisoptera N.	1	—	—	7	10	9
Trichoptera	.1	1	—	5	4	8
Chironomid L.	21	23	16	39	50	40
Gastropoda	38	36	32	18	9	20
			Year-class VI–VIII			
Amphipoda	.4	—	—	21	26	15
Isopoda	25	21	27	18	16	21
Ephemeroptera N.	4	7	3	7	2	3
Anisoptera N.	12	10	14	4	3	3
Trichoptera	1			3	5	5
Chironomid L., P	15	11	19	3	7	4
Gastropoda	38	49	33	40	38	46

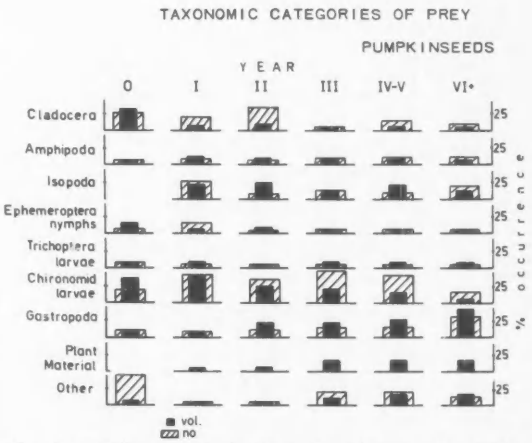

FIG. 2. Major taxonomic categories of prey eaten by the different year-classes of pumpkinseeds; mean annual figures based on combined May–November data.

diet of the larger fish. Chironomid larvae and isopods are dominant foods throughout life. Amphipods, Ephemeroptera nymphs, and Trichoptera larvae are

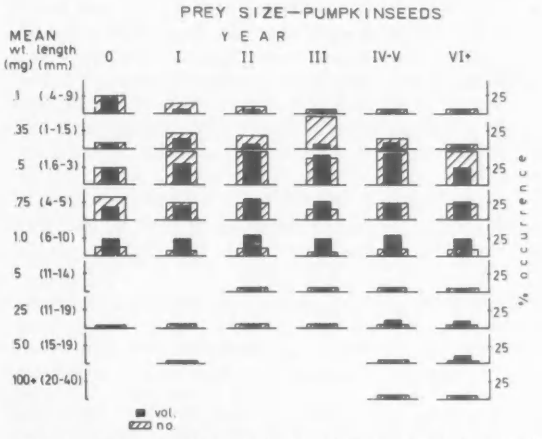

FIG. 3. Prey-size selection by the different year-classes of pumpkinseeds; mean annual figures based on combined May–November data.

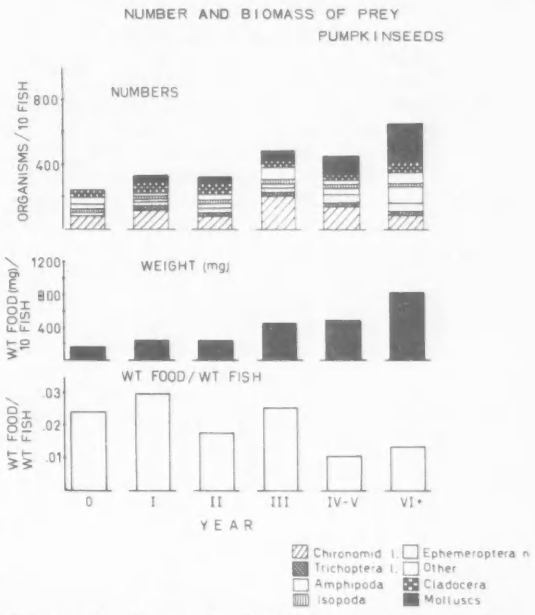

FIG. 4. Numbers and weights of major prey organisms, and ratios of stomach content weights to fish weight according to year-classes of fish; mean annual figures based on combined May–November data.

taken consistently but in smaller quantities. Prey items with mean weights ranging from 0.5 to 1.0 mg, and lengths of 1.6–10 mm predominate in the diet of pumpkinseeds and there is only a minor increase in average prey size with growth. Clearly this species is a generalist and the channeling of the age-classes towards different resources is not great.

Figure 4 shows that there is a progressive increase in total numbers and weight of organisms found in the stomachs of the fish even though there is a substitution of prey types (fewer chironomid larvae and more molluscs and Ephemeropera nymphs in the largest fish). Fish of Age IV and above consume less food relative to body weight than fish of Age III and under.

Table 3 shows the mean annual dietary overlaps between the year-classes (Levins (1968) index). They are based on 12 taxonomic and 10 prey-size categories of prey organisms. Overlaps are great even between the smallest and largest fish, being 0.6–0.8 for taxonomic categories of prey and 0.9–1.0 for prey size. The tables show the overlaps of pairs of year-classes on each other as reciprocals. Such values are likely to be different where one member is a generalist and the other a specialist, for in such cases the overlap of the former on the latter will be greater. Here, however, the reciprocal pairs are virtually the same, suggesting that both members are equally generalized in their feeding.

Seasonal variation in the diets of the age-classes — Table 2 shows month-to-month changes in the consumption of the major prey items by the various age-classes of fish. Rare or occasional prey items have been omitted. These include copepods, Anisoptera nymphs, and chironomid pupae in the case of Year I fish, and ostracods, Zygoptera nymphs, and adult Hymenoptera in Years II–VI. Other terrestrial organisms occasionally picked up by medium-sized and large pumpkinseeds included Lepidoptera larvae, spiders, oligochaetes, Orthoptera, and Megaloptera.

Since Year 0 fish only moved into the weed beds of Cow Island Bay in August (Keast 1977c), diet data are only detailed for the autumn. Although chironomid larvae were the dominant prey item, there was a replacement of isopods and ostracods by Cladocera and Ephemeroptera nymphs in October. These, and the Year I–III fish, vacated the inshore areas for deeper waters in October.

Diets of the Year I–VII fish when viewed on a month-to-month basis show that while a few kinds of prey are eaten regularly, the utilization patterns of most food items fluctuate considerably in the course of the season. Thus, chironomid larvae consistently formed a major part of the diet in Year I fish, exceeding 25% of stomach content volumes in all months except September, as did gastropods in the Year VI-VII fish. Highly seasonal items included amphipods. Note their occurrence in the diet of the Year I and II fish in September.

Table 2 also provides insight into the mechanism whereby one food type is replaced by another during growth. This occurs not by a consistent decrease or increase but by being a major prey item in fewer, or more, months. The utilization of chironomid larvae by the different year-class groups provides an example. In Year I and II fish they were a major item (exceeding

10% of mean stomach content volumes) in 5 mo, Years IV–V in 3 mo, and Years VI–VII in only 1 mo. The reverse pattern is seen in the progressive increase in gastropod consumption with increasing age.

The explanation for these shifts presumably is that the growing fish switch to prey of optimum size; the shift, however, is modified by the size of the prey organisms available at the time. Note, for example, that in Year II fish, 70 isopods make up 28% of the stomach content volume in May but that 42 account for only 8% in August.

Figure 5 shows month-to-month fluctuations in the numbers and biomass of six major food items of pumpkinseeds: chironomid larvae, isopods, the amphipod *Hyalella azteca*, Anisoptera nymphs, Trichoptera larvae, and gastropods. Figure 6 shows seasonal changes in body size in Anisoptera nymphs and Trichoptera larvae.

Explanation for some of the major seasonal dietary shifts now becomes apparent:

a) Isopods had weight peaks in May and October, and a numerical one in late May–early June, the latter representing the first major hatch. Isopods made up the

TABLE 2. Food of *Lepomis gibbosus*, Years 0–VIII, Lake Opinicon. Numbers of main food organisms per 10 fish and percentages of total volumes in the stomachs. N = nymph; P = pupae; L = larvae. *N* = number of fish sampled.

	May		June		July		August		September		October		November
Food organisms	No.	% Vol	No.	% Vol	No.	% Vol	No.	% Vol	No.	% Vol	No.	% Vol	No. % Vol
							Year 0 fish						
N									*61*		*64*		
Cladocera	not		not		not		not		11	8	67	30	not
Copepoda	present		present		present		present		12	5	18	10	present
Ostracoda									13	20	8	1	
Amphipoda									5	1	8	4	
Isopoda									24	16			
Ephemeroptera N.									2	1	2	13	
Anisoptera N.									2	4			
Chironomid L.									42	32	32	34	
Chironomid P.									7	5			
Hydracarina											152	4	
Others									1	8	7	4	
							Year I fish						
N	*72*		*65*		*74*		*67*		*70*		*65*		
Cladocera	95	7	29	7	42	10	5	1	50	8	47	13	
Ostracoda	2		4	2	66	4			1				
Amphipoda	27	3	15	2	4	6	30	10	23	18	26	9	not
Isopoda	131	36	41	12	4	4	40	11	146	33	43	23	present
Ephemeroptera N.	5	8	13	12	4	11	35	22	7	14	4	10	
Trichoptera	4	3	16	6	12	3			5	12	10	4	
Chironomid L.	168	36	129	46	134	45	95	46	73	13	68	26	
Gastropoda	29	4	22	12	65	15	20	3	5	1	5	2	
Pelecypoda					3	1					12	4	
Seeds							4	4			28	8	
Others	18	3	5	1	2	1	5	3	1	1	19	1	
							Year II fish						
N	*69*		*68*		*62*		*46*		*64*		*65*		
Cladocera	49	6	5	6	6	1			592	21	12	3	
Amphipoda	1	1	17	1	15	9	37	6	53	26	30	5	
Isopoda	70	28	17	7	6	2	42	8	20	17	92	52	
Ephemeroptera N.	6	6	10	14	10	8	7	4	8	5	3	2	
Anisoptera N.							3	7	3	1			
Trichoptera L.	4	1	22	3	2	1	7	5	10	7	22	5	not
Chironomid L.	233	16	373	36	367	43	143	44	25	9	112	10	present
Chironomid P.	2	1			1	1	10	2					
Other Diptera L., P	2	1			3	3					2	2	
Diptera A.	1	2			2	1	2	1					
Coleoptera L., A	1	3			2	1					4	1	
Gastropoda	50	33	88	34	65	26	43	19	20	9	8	2	
Pelecypoda					8	3			13	5	28	6	
Seeds											42	10	
Others	21	2	127	2	4	1	10	4			2	2	

TABLE 2 (*Concluded*)

	May		June		July		August		September		October		November	
Food organisms	No.	% Vol	No.	% Vol	No.	% Vol	No.	% Vol	No.	% Vol	No.	% Vol	No.	% Vol
							Year III fish							
N	*64*		*72*		*96*		*78*		*74*		*65*			
Cladocera	78	3	13	2	32	1	2	1	25	2	8	1		
Amphipoda	32	2	26	4	19	7	10	6	28	4	73	11		
Isopoda	82	17	82	8	15	7	15	1	18	3	107	36		
Ephemeroptera N.	6	5	18	10	1	2	4	10	8	2	7	3		
Anisoptera N.	2	2	1	6	3	1	5	17	6	5	7	3	not	
Trichoptera	14	1	11	4	12	5	14	10	29	20	9	3	present	
Chironomid L.	634	35	89	27	273	31	51	7	23	3	108	10		
Chironomid P.	8	5	17	3	19	4	8	3						
Diptera A.	11	4	4		3	1	4	1	6	1				
Coleoptera	.6	.5	1	1	4	3	5	11	6	.2				
Gastropoda	88	16	99	27	84	28	61	18	18	27	8	4		
Pelecypoda	26	5	2	3	7	4			3	7	35	7		
Plant remains	.6	.5		5				1		8		1		
Seeds	.6		2				144	14	156	18	114	21		
Others	8	4												
							Year IV–V fish							
N	*75*		*74*		*82*		*102*		*104*		*102*		*71*	
Cladocera	108	1	11	2	2	1	18	1	3	2	19	1	258	20
Amphipoda	20	1	11	2	29	6	35	6	26	8	53	8	112	9
Isopoda	57	46	25	12	15	5	36	6	23	16	86	32	28	11
Ephemeroptera N.	14	1	5	4	5	3	5	4	2	1	2	1	28	6
Zygoptera N.	5	1	3	11	3	5	1	2	7	1	1	1	1	1
Anisoptera N.	5	1	5	2	3	3	4	12	2	8	1	7	7	6
Trichoptera	12	3	2	3	11	4	9	8	2	8	9	4	39	16
Chironomid L.	493	18	176	12	230	23	88	9	34	6	60	6	24	2
Chironomid P.	1	1			28	8	1	2			2	2		
Diptera A.	11	1	4	2	1	2	1	2	1	2			3	3
Coleoptera L.			3	1	1	.5	1	2	1	2	7	3	10	14
Gastropoda	58	23	96	35	59	31	66	33	58	23	51	15	5	9
Pelecypoda	3	1			5	1			7	4	49	5		
Plant remains		1		5		7		6		9				2
Seeds							25	2	159	7	63	14		
Others	7	1	6	9	2	.5	5	5	8	2	23	1	45	1
							Year VI–VIII fish							
N	*71*		*62*		*65*		*61*		*65*		*67*		*33*	
Cladocera	6	3			3	3	14	6	2		18	2	284	17
Ostracoda			3								97	6	37	1
Amphipoda	21	4	24	1			280	20	48	8	92	8		
Isopoda	47	22	123	20	23	7	130	17	33	4	25	19	39	8
Decapoda			1	6					3	14				
Ephemeroptera N.	3	4	36	4			11	4	10	9	13	4	18	3
Anisoptera N.	7	11	9	10			4	4	10	21	15	19	4	9
Trichoptera	5	1	6	2	16	15	14	5	26	3	3	3	37	18
Chironomid L.	126	3	52	5	157	10	26	4	56	1	85	6	19	4
Chironomid P.	22	9			147	10								
Hymenoptera		2	2		3	1								
Coleoptera L.		2	5						4		2	1	18	12
Gastropoda	142	38	278	50	96	38	210	39	164	29	32	28	24	26
Pelecypoda					17	2							4	2
Plant remains						6		1		8		2		
Others	1	1	7	2	6	8			1	3	2	1		

largest percent of the total pumpkinseed diet in May and October.

b) Amphipods were not important in the diet of pumpkinseeds except in the case of age-groups I and II in September when they made up 34 and 26% of the stomach content volumes, respectively. This was correlated with a striking amphipod population buildup at this time (Fig. 5).

TABLE 3. Mean annual dietary overlap between year-classes of pumpkinseeds (Levins index). The first figure represents α_{ij}, and the second figure, α_{ji}. Year 0 fish, only present in the study area in September–October, are not shown.

Based on 12 taxonomic categories

		(j)			
		II	III	IV–V	VI+
(i)	I	.87/.83	.97/.78	.89/.79	.64/.87
	II		.86/.72	.90/.83	.58/.82
	III–I			.91/1.01	.54/.91
	IV–V				.64/.97

Based on 10 pre-size-categories

		(j)			
		II	III	IV–V	VI+
(i)	I	1.05/.94	1.02/.77	1.09/.85	.92/.94
	II		.92/.78	1.06/.92	.91/1.04
	III–I			.74/.82	.63/.85
	IV–V				.86/1.14

c) Anisoptera nymphs were generally too large to form a major dietary item for most age-groups of pumpkinseeds. They were, however, of modest importance to Year III and IV–V fish in August at a time when small-bodied, newly hatched nymphs became abundant (Fig. 6). The large Year VI–VII fish, in contrast, were less constrained by size considerations, taking significant numbers in May, June, September, and October. They were absent from the diet in July when numbers of the nymphs are low with only large preemergent ones remaining.

d) Ephemeroptera nymphs were eaten in moderate quantities throughout the summer but in August there was a jump in consumption by the Year I fish when they made up 22% of the stomach content volume and averaged 35 individuals per 10 stomachs. There was a marked late-summer numerical buildup with a predominance of small-bodied, newly hatched Ephemeroptera nymphs present then (see Keast 1977b fig. 8 and 9).

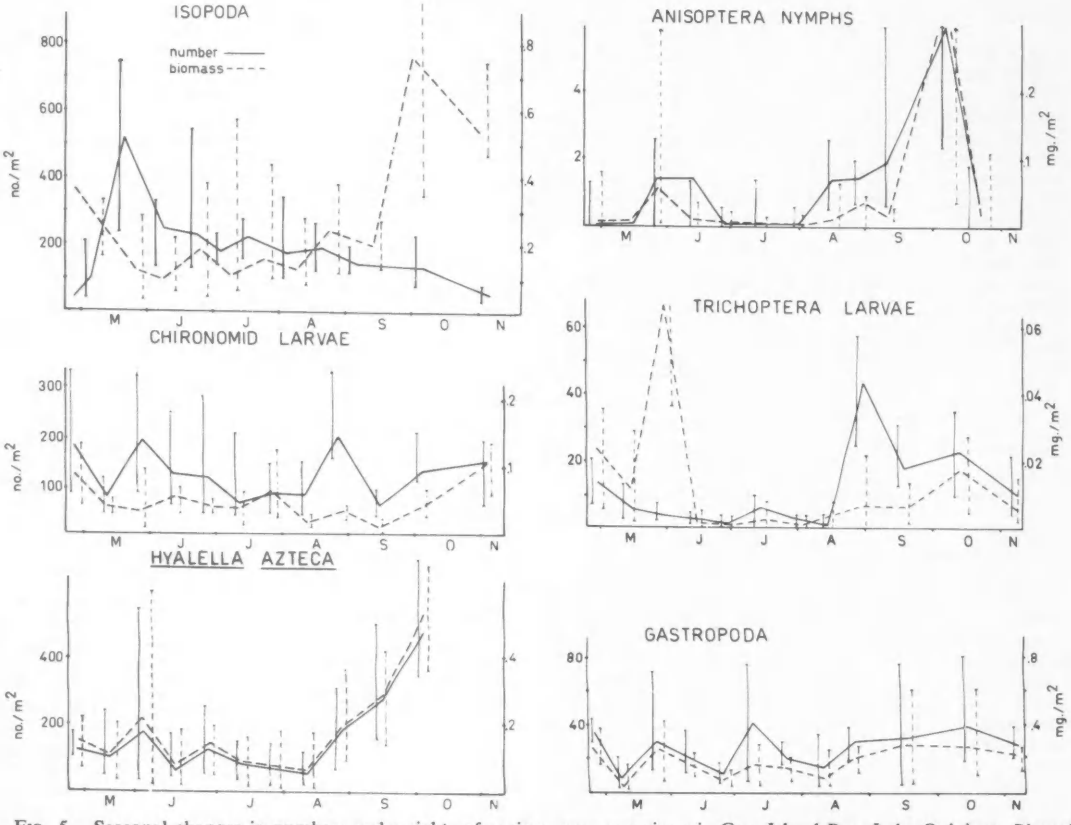

FIG. 5. Seasonal changes in numbers and weights of various prey organisms in Cow Island Bay, Lake Opinicon. Plotted as of medians and interquartile ranges.

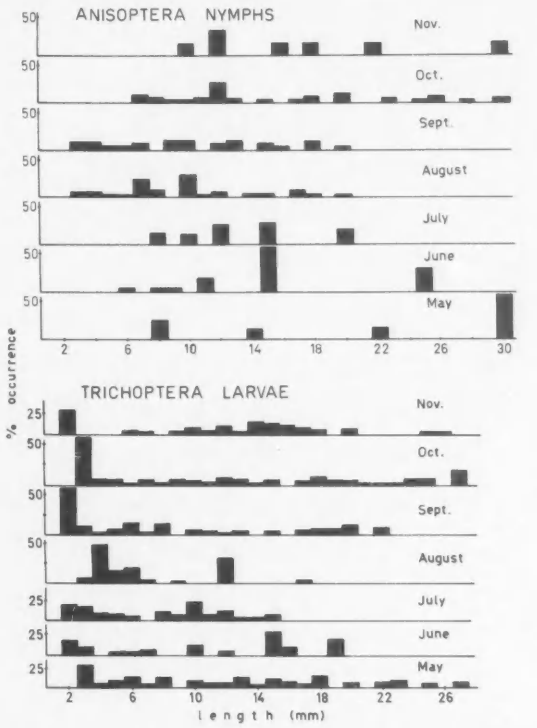

FIG. 6. Seasonal changes in size (length) distribution of two major prey types, Cow island, Lake Opinicon.

e) Trichoptera larvae formed a significant part of the diet of Year I, II, and III fish in September and of Year IV–VII fish in November. The latter, which are not limited by size constraints, took them also in July. Trichoptera larvae in the benthos peaked numerically in August and September, with the numbers remaining fairly high in October and November (Fig. 5). Small-bodied larvae predominated in September (Fig. 6).

f) Molluscs were taken by Year I fish only in July (65 per 10 stomachs). This was linked to the major summer hatch of three of the most important food species, *Amnicola limosa*, *Gyraulus parvus*, and *Valvata tricarinata* (Turnbull 1975), which were small bodied and presumably soft shelled at this time. Note the small numerical peak in July in Fig. 5. Pumpkinseeds in Lake Opinicon consumed molluscs only up to a shell width of 5 mm (Harker 1976), which are abundant all summer. Pumpkinseeds are the only major predator of molluscs.

g) Chironomid larvae were the most consistently harvested prey item. Though showing minor numerical peaks, this resource is available throughout. The diversity of genera present (at least 20 in the study area) and their differing population cycles explain this.

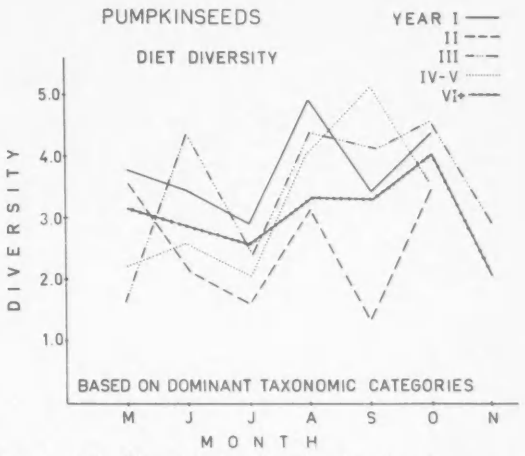

FIG. 7. Monthly shifts in diet diversity, based on taxonomic categories of prey, in the year-classes of pumpkinseed.

Diversity and overlap in the year-class diets — Monthly variation in mean diet diversity (Levins (1968) formula) in the various age-class groups, in terms of taxonomic categories of prey, is shown in Fig. 7. The diet of Year II is the least diversified and those of I and III the most. The main feature, however, is the striking seasonal fluctuations. There is general agreement in times of greatest and least diversity of diet in all year-classes.

Monthly diet overlaps of three year-classes (I, III, and VI–VII) on the others are shown in Fig. 8. Two features are apparent: (1) overlap values fluctuated greatly from month to month and (2) there was a good general agreement in times of maximum and minimum overlap.

Various year-classes tended to concentrate on particular resources at specific times (Table 2). For example, note the predation on isopods in May and October and amphipods in the autumn. Since these resources peak in abundance, the overlaps do not mean an increase in potential competition between the year-classes but rather the reverse. Note, however, that the month of greatest diet overlap (July or June–July) is a time of lowest populations of chironomid larvae, Anisoptera and Ephemeroptera nymphs (Fig. 5 and Keast 1977b) due to emergence, and fairly low numbers of *Hyalella*, isopods, and Trichoptera larvae (Fig. 5).

Feeding Ecology of Bluegills and Pumpkinseeds

Feeding data on the diets of the year-classes of the bluegill, developed simultaneously with the present study, is published elsewhere (Keast 1977a). The two species have similar year-class compositions, but because of the later spawning of the bluegill, this species

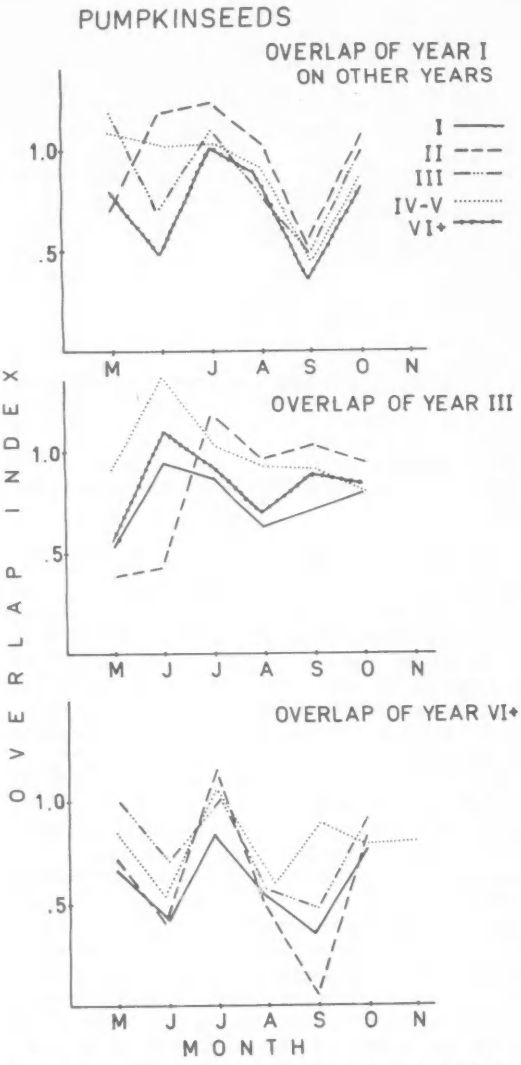

FIG. 8. Seasonal changes in diet overlap of three age-class groups, I, II, and VI–VIII on the other year-classes, May–November. Degree of overlap varies but times of greatest and least overlap tend to correspond.

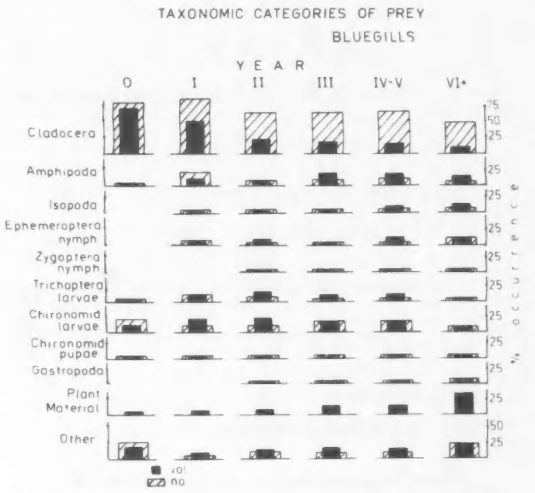

FIG. 9. Major taxonomic categories of prey eaten by the different year-classes of bluegills; mean annual figures based on combined May–November data.

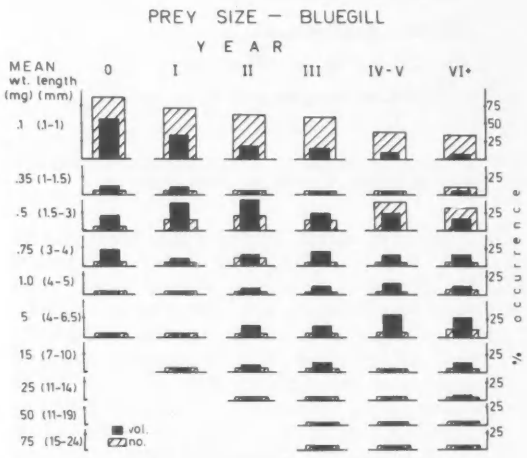

FIG. 10. Prey-size selection by the different year-classes of bluegills; mean annual figures based on combined May–November data.

averages smaller in its earlier years. Relative length ranges at the end of the summer's growth period were as follows: Year 0, 24–42 mm (35–52); I, 47–68 mm (52–76); II, 66–93 mm (76–98); III, 92–108 mm (94–118); IV, 106–123 mm (115–137); V, 117–137 mm (128–151); VI, 134–156 mm (145–167); VIII, 147–167 mm (162–180).

A mean annual overview of the dietary differences between the age-classes of bluegills shows that in terms of taxonomic and prey-size categories, the diets of the year-classes are substantially the same (Fig. 9 and 10). Consumption of chironomid larvae and pupae, Trichoptera larvae, and amphipods varies little with age. Cladocera, the most important single food, shows a declining importance with age, accounting for 75% of the numbers and 70% of the total volume of prey in Year 0 but falling to 45% by number and only 10% of the volume in Years VI–VII. The loss is in part compensated for by a greater consumption of plant material by the larger fish. The bulk of bluegill prey consists of organisms of body length 0.1–6.5 mm and

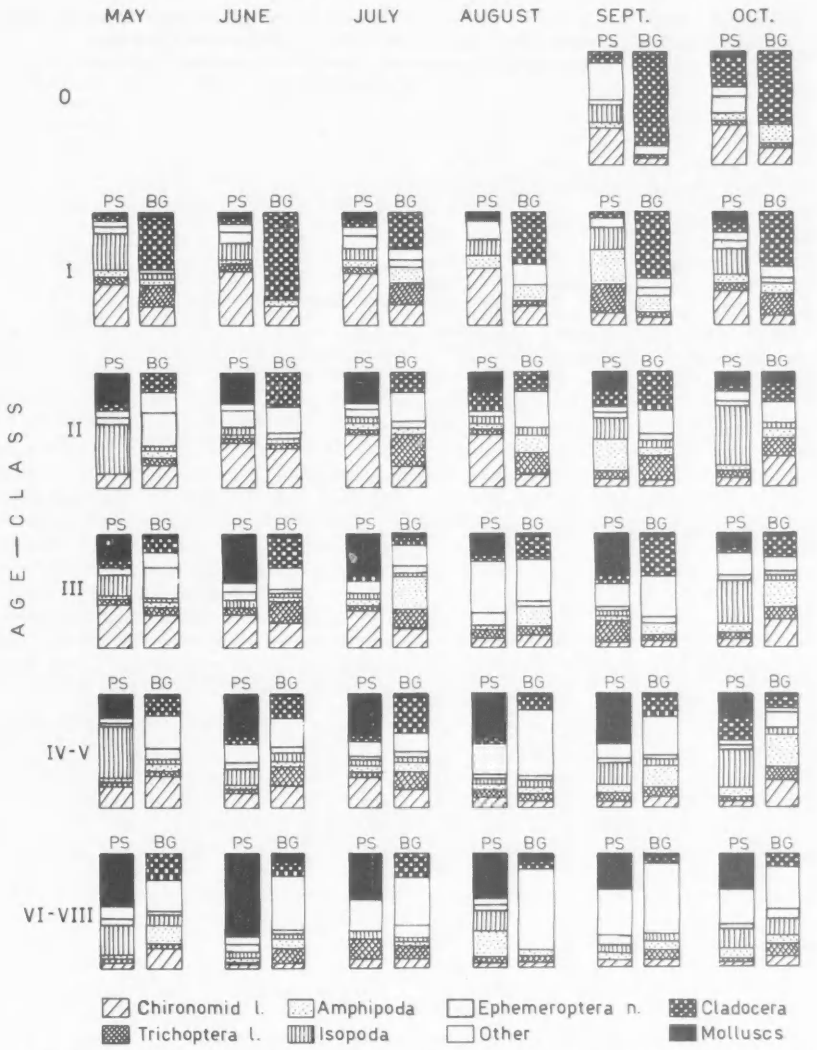

FIG. 11. The diets of pumpkinseeds (PS) and bluegills (BG) compared. Percent volumes of the different prey types eaten by corresponding age-classes of the two species from May to October. Year 0 fish, which hatch in June, do not occur in the shallow-water weed beds until late summer.

mean weight of 0.1–5.0 mg. The bluegill, like the pumpkinseed, is a generalist feeder.

Diets of the two species basically differ in the greater consumption of Cladocera by the former and molluscs and isopods by the latter (cf. Fig. 2 and 9). The bulk of the pumpkinseed feeding is on items rather larger than those of the bluegill (cf. Fig. 3 and 10).

Seasonal diets of the various age-classes in the two species are contrasted in terms of the percent volumes in Fig. 11. The differences are as follows:

Year 0 fish. In their first autumn bluegills ate mainly Cladocera, whereas pumpkinseeds took proportionately more chironomid larvae as well as isopods in September and Ephemeroptera nymphs in October.

Year I fish. In all months Cladocera dominated the diet of the bluegills, which took proportionately less chironomid larvae than pumpkinseeds, but more Trichoptera larvae in May, July, and October. The pumpkinseeds took consistently more isopods and Ephemeroptera nymphs. Month-to-month fluctuations

TABLE 4. Mean annual dietary overlap between equivalent and adjacent classes of pumpkinseeds and bluegills. The first figure represents α_{ij}, the second figure, α_{ji}.

PUMPKINSEED (*i*)	BLUEGILLS (*j*)					
Based on taxonomic categories (numbers)						
	0	I	II	III	IV–V	VI–VIII
I	1.13/.61	.64/.30	.70/.30			
II		1.01/.55	1.11/.55	1.13/.64		
III			.65/.40	.40/.31	.38/.29	
IV–V				.55/.47	.53/.45	.43/.46
VI–VIII					.63/.25	.62/.28
Based on prey-size categories (numbers)						
	0	I	II	III	IV–V	VI–VIII
I	1.20/.16	.59/.21	.65/.34			
II		.45/.23	.47/.34	.47/.34		
III			.34/.24	.28/.24	.52/.56	
IV–V				.36/.30	.71/.45	.56/.82
VI–VIII					.78/.62	.61/.68
Based on taxonomic categories (volumes)						
	0	I	II	III	IV–V	VI–VIII
I	.76/.51	.61/.37	.74/.75			
II		.51/.28	.64/.59	.64/.62		
III			.59/.57	.57/.59	.62/.70	
IV–V				.57/.59	.62/.71	.65/.83
VI–VIII					.29/.50	.38/.73

in the diets of the two species did not entail a significant departure from these basic differences. Some of the diet fluctuations, such as reduced consumption of chironomid larvae by bluegills in July and increased consumption of amphipods by the pumpkinseeds in September, can be correlated with changes in the abundance levels of these organisms in the environment (Fig. 5).

Year II fish. Consumption of Cladocera by the bluegills is now less but is compensated for by increased Trichoptera larvae consumption from July onwards and the utilization of several different food types that temporarily become abundant — see "other" category in Fig. 11 and tabulations in (Keast 1977a). These are Diptera adults (mostly small chironomids picked up at emergence) in May, nonchironomid Diptera larvae in June, Hydracarina and seeds of aquatic plants in July, Diptera adults in August, and a diversity of items in September and October. By contrast, in the pumpkinseeds, molluscs become a major diet component and isopods are important in May, September, and October.

Years III–IV fish. The same general levels of difference and preference characterized these bigger fish. In August and September the bluegills took more *Najas* seeds and plant remains than pumpkinseeds.

Years VI–VIII fish. In pumpkinseeds, molluscs made up a major segment of the diet in all months, and isopods were important in May, August, and October. Bluegills, by contrast, although still taking some cladocerans, now ate much plant material in summer and autumn along with adult Diptera and Hymenoptera. Pumpkinseeds took less vegetable material than bluegills but ate Anisoptera nymphs in September–October (Table 2).

Month-to-month comparison of the diets of the year-classes of the two species shows that (1) the basic dietary differences indicated by the mean annual figures apply despite fluctuations in amounts of favored items eaten; (2) foods common to the diets of the two rarely exceeded 35% by volume; (3) less important items shared were usually not harvested to an equal extent by the comparable year-classes of the two species; and (4) diets changed with age in both species but in different ways.

Dietary overlaps — The critical component in any consideration of species interaction is, of course, that segment of the resource base being shared or competed for by both. As Fig. 11 shows, pumpkinseeds and bluegills take significant amounts of chironomid larvae. However, Trichoptera larvae, amphipods, isopods, and Ephemeroptera nymphs important food to one species are eaten only in small amounts or intermittently by the second.

Mean annual diet overlap values for taxonomic and size categories of prey organisms for bluegills and pumpkinseeds of equivalent and adjacent age-groups are given in Table 4. They are based on numbers of organisms. Most are relatively high (0.6–1.1). Less expected is the marked difference in many of the reciprocal pairs of values. Thus, in all calculations involving the Year 0, I, and II fish, the overlap values of pumpkinseeds on bluegills is much greater than the

TABLE 5. Monthly variation in diet overlap between equivalent age-classes of pumpkinseeds and bluegills based on volumes of different taxonomic categories of foods in the stomachs. The first figure represents the overlap of pumpkinseeds or bluegills, and the second figure, the overlap of bluegills on pumpkinseeds.

	Year				
	I	II	III	IV–V	VI–VIII
May	.42/.37	.16/.22	.66/.67	.31/.37	.22/.36
June	.55/.25	.69/.56	.47/.48	.31/.51	.16/.66
July	.57/.72	.37/.42	.44/.54	.36/.33	.38/.58
Aug.	.26/.13	.32/.55	.53/.29	.31/.52	.32/.32
Sept.	.51/.23	.65/.56	.20/.24	.51/.52	.35/.55
Oct.	.66/.43	.22/.30	.54/.33	.51/.51	.50/.74
Nov.				.31/.75	.33/.22

reverse. There are no such differences in Year III–VIII fish. The reason for the disparity lies in the very large numbers of Cladocera consumed by bluegills. Diversity indices, which are incorporated in the overlap formula, reflect two features, numbers of different prey types and evenness of spread of these in the diet. The numerical predominance of the small-bodied Cladocera in the bluegill diet perhaps introduces a bias making it appear more of a specialist than it actually is. To compensate for this a third set of calculations based on volumes rather than numbers of organisms is also given (see bottom of Table 4). Overlap values between the pairs of reciprocals now become rather more even. Overlap values of pumpkinseed on bluegills are still the higher in the case of Year 0 and I fish, but not in the Year II.

Monthly diet overlap values are of more immediate significance than the mean annual ones. These are given for corresponding year-classes of pumpkinseeds and bluegills (volumes of each taxonomic category) in Table 5. Year 0 fish are not included, since data for them are only available for September and October. Two conclusions are evident: (1) When viewed on a month-to-month basis overlap values are much less than when the data is grouped as mean annual figures; most are in the range of 0.3–0.6 with four pairs of reciprocals being less than this and in six one or the other values exceeding it. Mean annual figures thus exaggerate the degree of overlap. (2) In Year II–VIII fish the overlap values between the pairs of reciprocals are usually the same (19 cases). However, the overlap of pumpkinseeds on bluegills is markedly greater than the reverse in 3 cases, and the reverse is true in 4 (Table 5).

The reasons for these disparities become apparent from a comparison of Table 2 and the bluegill food data in Keast (1977a). The overlap of pumpkinseed on bluegill diet is markedly the greater in Year I fish in June and September (Cladocera make up 61 and 52% by volume of the diet of the latter) and Year III fish in October (isopods account for 36% of the bluegill diet). In the Year IV–V fish in June and Years VI–VII in July the reverse is the case; the pumpkinseeds appear as the specialist feeder (molluscs make up 69 and 34% of their diet, respectively).

Relationship between body morphology and prey differences — While pumpkinseed and bluegill are of similar size, body shape, mouth size, and gill raker morphology, they differ in one significant feature, the structure of the pharyngeal teeth. The pharyngeal pads of pumpkinseeds are covered with large molariform teeth that form efficient crushing plates (Hubbs and Lagler 1964), while those of bluegills are equipped with fine needlelike teeth. The former preadapt their owner to feeding on molluscs and other heavier bodied

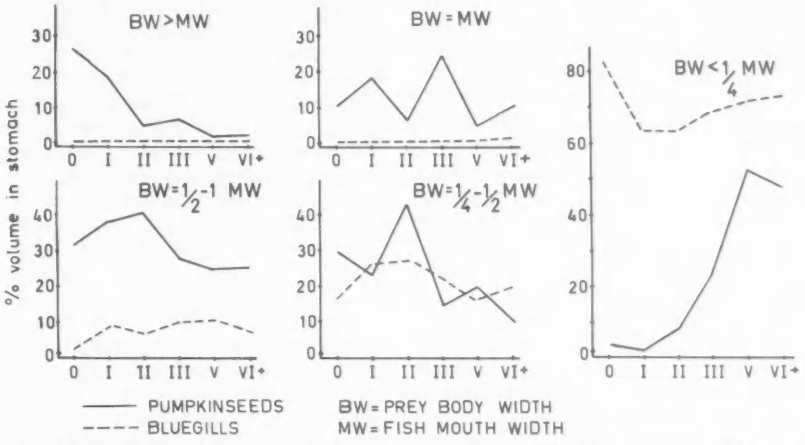

FIG. 12. Prey-size consumption, using prey body width as the criterion relative to internal mouth width of fish; various year-classes of pumpkinseeds and bluegills compared. Graph shows that the percent prey in which the prey width exceeds the mouth width is between half the mouth width and the mouth width, etc.

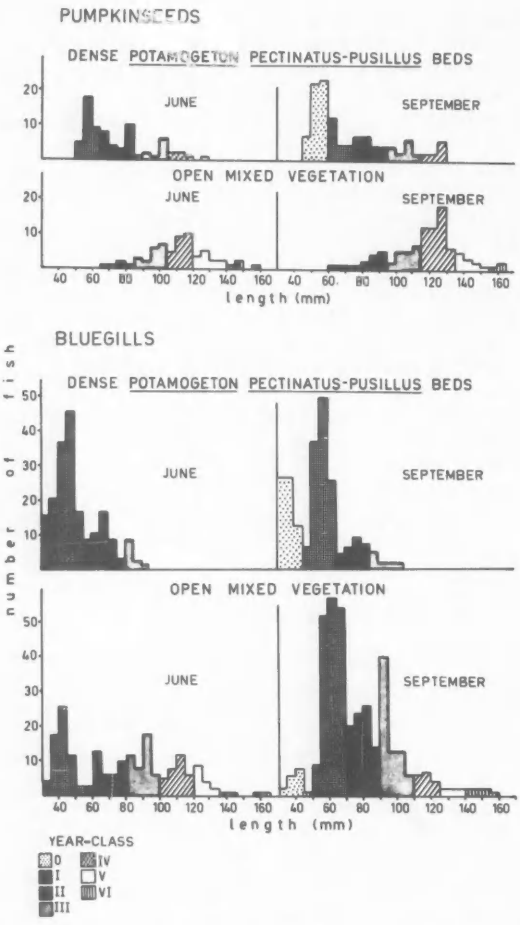

FIG. 13. Distribution of the different age-classes of pumpkinseeds and bluegills in two vegetation types in Cow Island Bay: the dense *Potamogeton pectinatus–P. pusillus* beds and the open mixed plant community consisting of scattered *P. richardsonii–P. robbinsii–P. amplifolius, Myriophyllum, Ceratophyllum, Elodea*, and others.

prey. Accordingly, pumpkinseeds selectively hunt for these prey types and this explains the month-to-month importance of molluscs in the diet. To the extent that their teeth are finer and more numerous, bluegills are morphologically channeled towards smaller prey or prey that does not need crushing, like Cladocera.

Since the mouth width is the same in individuals of similar body size in the two species, it is to be expected that even though pumpkinseeds of equivalent age are slightly larger than bluegills, they should select for prey of the same size. It has, however, been demonstrated that this is not so. In Fig. 12 this difference is put into an additional perspective, being viewed in terms of the width of the prey item relative to the inner mouth width of fish of different ages. While a considerable proportion of the pumpkinseed prey has a width greater than half its mouth width, an overwhelming percent of the bluegill prey has a width less than one quarter its mouth width. Bluegills, in other words, are utilizing prey of disproportionately small size. Note here arguments that selection of prey of small size is compensated for by reduced handling time where the prey is abundant and relatively sessile (Schoener 1971; Werner 1974; Werner and Hall 1976). However, Werner (1974) was able to experimentally demonstrate that bluegills when presented with particles of chopped kidney of varying sizes showed preference for those in which the prey width to mouth width ratio was 0.59, regardless of the size of the fish.

Habitat relationships in Lake Opinicon — If the equivalent age-classes of bluegills and pumpkinseeds have different habitat utilization patterns, then the 0.3–0.6 diet overlap values indicated in Table 5 may not be a true index of the level of ecological interaction between these two species. Accordingly, habitat relationships must be considered.

Both species are lake margin dwellers in Lake Opinicon, and throughout the summer the bulk of the biomass of both species is concentrated into waters of 2.5 m depth or less. This can be linked to the spatial distribution of the benthic invertebrates (Keast and Harker 1977). These sunfishes spend the winter in deeper waters. Bluegills concentrate inshore in mid-May but most pumpkinseeds not until June (Keast et al. 1977). There is a pronounced drift from the shallower sections into waters 2–3 m deep in August–September. The last of the smaller fish leave the margins in October, but a few large fish of both species remain there until the beginning of November.

There are some differences in spawning sites in the two; pumpkinseed nests are rather scattered, those of bluegills are concentrated into dense colonies that are commonly in deeper water. Pumpkinseeds usually spawn ahead of the bluegills e.g. June 7–30 compared to June 25–July 15 in 1969–70 (Amundrud et al. 1974). Hatchlings of both species share a common inshore open-water habitat, although as a result of the differences in spawning dates, occupation is largely sequential (Keast 1977c). The young of both species move to dense inshore weed beds at the age of 3–4 wk; the main habitat in Cow Island Bay is the dense *Potamogeton pusillus–P. pectinatus* beds at the head of the bay. Such an inshore movement at this age has also been described in Crane Lake, Indiana, by Werner (1969).

Habitat utilization patterns in pumpkinseeds and bluegills of different ages are shown in Fig. 13 and Table 6. Year 0 and I pumpkinseeds are largely confined to the *P. pectinatus–P. pusillus* beds, and the bulk of the Year II are also here. In contrast, the larger fish, although rather generalized in their habitat preferences, avoided the denser weeds and were common in lightly weeded and open mixed vegetation types. Similar differ-

TABLE 6. Percent occurrence of bluegill (BG) and pumpkinseed (PS) age-classes in different habitat types in June, Lake Opinicon

Year-class of fish	I		II		III		IV–V		VI–VIII	
	BG	PS	BG	PS	BG	PS	BG	PS	BG	PS
Potamogeton pusillus–P. pectinatus	52	100	38	98	28	71		11		2
Weedy shallow	40		55	2	12	14	25	28	16	28
Weedy deep	8		7		27		23	1	20	2
Mixed bottom					12	6	13	18	14	18
Sand and clay					5	2	13	26	12	32
Gravel					5	3	11	15	12	12
Rocks and rocky margin					5	4	10	11	16	5
Open water offshore					6	1	5	1	10	1

TABLE 7. Habitat and food overlap between equivalent age groups pumpkinseeds (*i*) and bluegills (*j*) for June, Lake Opinicon.

		Age-classes of fish				
		I	II	III	IV–V	VI–VIII
Habitat overlap	$\alpha 1_{ij}$	.42	.32	.34	.64	.55
	$\alpha 1_{ji}$	.88	.75	.82	.62	.68
Food overlap	$\alpha 2_{ij}$	.55	.69	.47	.31	.16
	$\alpha 2_{ji}$	.25	.56	.48	.51	.66
Means of $\sum \alpha 1_{ij} + \alpha 2_{ij}$		.48	.51	.40	.48	.36
$\sum \alpha 1_{ji} + \alpha 2_{ji}$		.57	.62	.65	.66	.66
Average		.53	.57	.52	.57	.53

ences are found in the bluegills. However, in this species there are also large numbers of Year 0–II in the areas of more open weed. This is presumably a consequence of the higher populations of bluegills.

Preferred habitats of the older pumpkinseeds and bluegills along the lakeshore margin, as determined by strip counts (Keast et al. 1977), show that adult pumpkinseeds are most abundant in the areas of sandy shallows. Quantitative analysis of the distributions of older bluegills (Keast 1977a) shows that while they occur virtually everywhere, considerable aggregations of Year IV–V fish occur in the open waters offshore an area from which the pumpkinseeds are virtually absent. Year VI–VIII fish are prominent in areas of rocky shoreline.

Werner et al. (1977) found a consistent difference in the level in the water column at which bluegills and pumpkinseeds swim: 99% of the former were encountered close to the bottom, whereas the latter were found well up in the water column. Vertical separation of this kind also characterizes young in the first weeks of life (Werner 1966). Feeding observations of mixed aggregations of the two species in lightly weeded sandy areas in Lake Opinicon have shown pumpkinseed feeding mostly from the bottom and bluegills from the vegetation (July–August). In vegetation-free rocky areas, although bluegills do some bottom feeding, they tend to keep well up in the water column and readily rush prey that strike the surface. A level of separation in the height at which the two species forage is thus also indicated for Lake Opinicon.

Table 7 incorporates diet and habitat data on the two species to produce combined ecological overlap figures. It is calculated as "summation alpha," representing the mean of the sum of the two values, these two niche dimensions not being independent (Cody 1974). The resultant overlap figures, 0.52–0.57 on the Levins scale, are not very different from those for diet overlap alone. These figures are only slightly different from those obtained by other workers for co-occurring species, e.g. 0.56 for niche overlaps in grassland birds (Cody 1968), 0.51 among blackbird species in Washington (Orians and Horn 1969), and 0.47 for Australian lizards (Pianka 1969) (the different formulae used by these workers only slightly influence these final figures).

A feature of the Lake Opinicon pumpkinseed–bluegill association is that both species enjoy striking numerical success. This argues that these calculated overlap figures based on two dimensions only are too high and that the real values are lower. It is, however, very difficult mathematically to bring more than two components into overlap calculations (Cody 1974). The reasons why these figures might be too high are as follows: (1) The two species, by tending to feed at different heights in the water column might, in part, obtain different species of chironomids, Trichoptera larvae, etc. (2) Since the food organisms were mostly identified only down to the order or family level, a very low level of resolution, differences might be obscured. (3) Pumpkinseeds tend to take slightly larger prey items than bluegills; they might, in part, be taking different genera. (4) Diet overlap levels fluctuate seasonally, sometimes falling to only 0.2–0.3. Both species are somewhat opportunistic in their choice of prey items and may move on to a common prey type that peaks in abundance. Increased diet overlap thus does not necessarily indicate increased interspecific competition. (5) Seasonal habitat changes involving shifts in abundance occur in both species. These are presumably linked to the occurrence of prey organisms. Such mo-

bility may help reduce interactions between the species. (6) The prey items shared by both species may not always be sought by both species simultaneously. Harker (1976) found cases of pumpkinseeds moving on to an abundant resource at one time but not when it peaked again later in the season.

There is now a considerable literature on ecological overlaps between cohabiting species with accompanying discussions as to how the results should be interpreted (e.g. MacArthur and Levins 1967; Levins 1968; Orians and Horn 1969; Pianka 1969; May 1974; Cody 1974). Because it is impossible to simultaneously calculate multiple parameters of interaction, most authors have only developed figures for two parameters. Authors agree that, theoretically, there must be an upper limit to the amount of ecological overlap that can occur between species. Nobody has, however, demonstrated just what that is. Again it may well be that poikilotherms that do not need to feed continuously may be able to accept higher levels than can co-occurring homeotherms.

Discussion

Pumpkinseeds and bluegills show dietary changes with age. Diet overlaps between the year-classes are marked but fluctuate from month to month. The two species exhibit basic differences in their major prey items and diet overlaps between equivalent and adjacent year-classes are only moderate. The differences can, in part, be linked to differences in the pharyngeal dentition that predispose the pumpkinseed to heavier bodied molluscs and isopods and the bluegill to soft-bodied Cladocera and chironomid larvae. There are some habitat differences between the two species but they are incomplete. When an overlap index is developed for diet and habitat combined, it proves to be not very different to that for diet alone.

A survey of the literature on feeding of pumpkinseeds and bluegills suggests that the ecological differences detailed here are also typical of the species in other parts of their range. Thus molluscs are recorded as a major item of pumpkinseed diet over much of its geographical distribution, e.g. Pearse (1918), De Ryke (1922), Ball (1948), and Ball and Tanner (1951). Seaburg and Moyle (1964) found that in two Minnesota lakes (Maple and Grove) gastropods made up 40 and 58% of the stomach content volumes and insects 54 and 22%, with gastropods dominating the diet for several weeks in midsummer. All studies have shown the importance of insects in the diet of pumpkinseeds. In Maple Lake, Minnesota, Ephemeroptera nymphs, chironomid, and Trichoptera larvae predominated, compared to chironomid and Trichoptera larvae in Grove Lake; small and large fish, however, consumed the same kinds of organisms (Seaburg and Moyle 1964). Chironomid larvae dominate the diet in Tuckahoe Creek, Virginia (Fleming and Woolcott 1966), and chironomids and isopods in Jones Creek, Ontario (Keast 1966). In both of these systems, molluscs are rare.

The considerable literature on bluegill feeding confirms that chironomid larvae, other small aquatic insects, and zooplankton, are the major diet components of this species (Moffett and Hunt 1943; Gerking 1954, 1962; Turner 1955; Fleming and Woolcott 1966). Plant material may be prominent in the diet (McCormick 1940; Rice 1941; Howell et al. 1941; Chable 1947; Gerking 1962) and is also taken by pumpkinseeds. Obviously the proportions of the different diet items eaten by both vary somewhat with the available resource base. Evidence that when molluscs are uncommon pumpkinseeds will consume higher proportions of chironomid larvae suggests that diet overlaps are going to be greater in some waterways than they are in Lake Opinicon. Obviously comparative feeding and habitat utilization studies on the two species in different waterways are needed.

Unfortunately there is no evidence as to how long bluegills and pumpkinseeds have coexisted in Lake Opinicon and in the Rideau system, but it is commonly believed that the bluegill, which tends to have a more southern distribution, is a later invader. Both, as "warmwater" species, must have originated in the Mississippi–Missouri system and entered the Great Lakes post-glacially from there. Presumably the basic morphological and ecological differences between the two species were evolved in the south, and hence, are not recent. Other ecological features, however, such as features of habitat utilization, may well be latter-day developments. The two species are so closely associated, moreover, that there is presumably little doubt that one is influencing the ecology of the other. Comparative studies in other water bodies are needed to see how basic some of the adaptations are and if they change under different sets of circumstances.

Acknowledgments

I extend my sincere thanks to the National Research Council of Canada for funding the present research and to the Queen's University Biology Station, Chaffey's Locks, Ontario, and its Director, Prof R. J. Robertson, for providing accommodation and research facilities.

I am also grateful to the students who helped net fish and accumulate the food data. The diagrams and overlap figures were developed by Miss J. Harker.

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Enhancement of Autotrophic Production by Nutrient Addition in a Coastal Rainforest Stream on Vancouver Island

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STOCKNER, J. G., AND K. R. S. SHORTREED. 1978. Enhancement of autotrophic production by nutrient addition in a coastal rainforest stream on Vancouver Island. J. Fish. Res. Board Can. 35: 28–34.

In 1976 streamside nutrient-enrichment experiments were conducted using wooden troughs. Tripling of the PO_4-P concentration, with or without a similar increase of NO_3-N, increased algal biomass on the troughs by 8 times after 35 days. Increasing NO_3-N alone had no appreciable effect on algal growth. A sloughing of algal biomass in August 1976 is believed to have been due to the instability of the heavy algal mat on the troughs and to the very poor light conditions that prevailed throughout August. Visual observation indicated that the relatively heavy algal population in Carnation Creek rapidly declined concurrent with the decline in the troughs. The percentage of diatoms in the algal assemblage remained the same in all troughs, and *Fragilaria vaucheriae* replaced *Achnanthes minutissima* as dominant on the phosphorus enriched trough. No shift to green or blue-green algal dominated assemblages occurred despite alteration of the N:P ratio. The dynamics of species succession, distribution, and growth, with and without nutrient addition, are discussed.

Key words: stream fertilization, autotrophic production, algal succession, N:P ratio, algal distribution, rainforest, algal biomass, diatoms, blue-green algae

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Nous avons poursuivi en 1976 des expériences d'enrichissement en éléments nutritifs d'un cours d'eau, utilisant des auges en bois. Le fait de tripler la teneur en PO_4-P, avec ou sans une augmentation semblable de NO_3-N, entraîne une augmentation de 8 fois la biomasse algale sur les auges après 35 jours. Une augmentation de No_3-N seul n'a pas d'effet appréciable sur la croissance des algues. La biomasse algale s'est détachée en août 1976, et on croit que l'instabilité de l'épais tapis d'algues sur les auges et les conditions lumineuses très pauvres qui ont prévalu en août en ont été la cause. L'observation visuelle indique que la population relativement lourde d'algues diminua rapidement dans le ruisseau Carnation en même temps que dans les auges. Le pourcentage de diatomées dans la communauté algale est demeuré le même dans toutes les auges, et *Achnantes minutissima* a été remplacé par *Fragilaria vaucheria* comme espèce dominante sur les auges enrichies de phosphore. Il n'y eut pas déplacement vers des communautés dominées par des algues vertes ou bleu-vert en dépit de l'altération du rapport N:P. Nous examinons la dynamique de la succession, de la distribution et de la croissance des espèces avec et sans addition d'éléments nutritifs.

Received May 30, 1977
Accepted September 7, 1977

Reçu le 30 mai 1977
Accepté le 7 septembre 1977

THE response of lake phytoplankton and periphyton to nutrient addition during the eutrophication process is well known, and factors responsible for changes in species succession have been identified (Lund 1969; Kalff et al. 1975; Schindler 1975; Stockner and Evans 1974). However, in rivers and streams the changes in algal production and species composition following nutrient addition are not adequately understood and factors responsible have not been adequately documented. Most studies have dealt with changes within and among communities in zones of influence of a pollution source, but relatively few studies attempt to relate observed temporal changes in periphyton communities to physical–chemical fluctuations immediately following application of nutrients.

From May to October 1976 nutrient enrichment experiments on stream periphyton using wooden troughs were conducted in Carnation Creek, a rainforest stream on the west coast of Vancouver Island. The studies were designed to test the impact of nitrate and phosphate additions (alone and in combination) on autotrophic

Printed in Canada (J4853)
Imprimé au Canada (J4853)

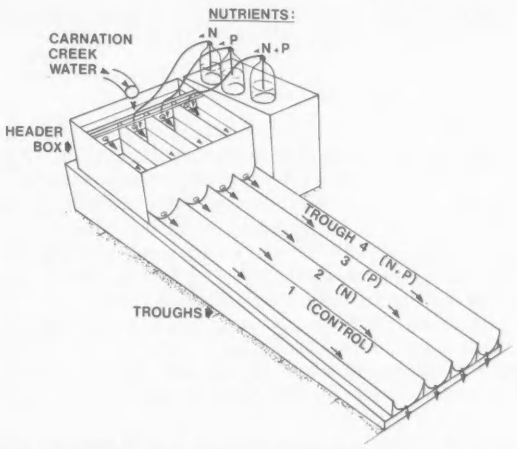

FIG. 1. Schematic representation of apparatus used for streamside nutrient-enrichment experiment.

production and species succession. Previous work had indicated that phosphate, not light, was limiting algal growth in Carnation Creek, but no detailed information on algal species succession and abundance in relation to enhanced nutrient concentrations accompanied this earlier work (Stockner and Shortreed 1976).

The main objectives of this work were to intensively study the response of attached algal communities to elevated nutrient concentrations and to determine the possibility of predicting the nature of the algal assemblage (blue-green, greens, or diatoms) following natural or man-induced nutrient enrichment in Pacific coastal oligotrophic streams. These results are particularly relevant to stream management strategies under consideration in British Columbia, which include the application of commercial fertilizer to streams as a possible means of salmonid fisheries enhancement on Canada's west coast.

Materials and Methods

In 1976 Carnation Creek water was piped into a header box, split into four equal chambers, and discharged into four identical wooden troughs, 4.25 m long, 9.0 cm wide, with 2.0-cm sides (Fig. 1). The troughs had 4 wk to establish an algal community prior to the commencement of nutrient additions.

Concentrated solutions of $NaNO_3$ and Na_2HPO_4 were prepared in 20 L carboys and continuously added to the header box chambers using capillary tubing (0.8 mm ID). From day 0 (June 25) to day 52 (August 20) (part A), trough 1 was the control; trough 2 nitrate only; trough 3 phosphate only; and trough 4 nitrate and phosphate combined. From day 52 (August 20) to day 101 (October 4) (part B), trough 1 was the control; trough 2 nitrate + EDTA; trough 3 phosphate + EDTA; and trough 4 was treated as a control to observe recovery.

The weekly sampling consisted of stopping water flow, scraping all accumulated algal growth from a measured undisturbed section of trough into a glass jar, and transporting the material immediately to the field laboratory. Contents of each jar were split into four equal portions using a circular plankton-splitting chamber. Two subsamples were put in glass jars with Lugol's acetate solution. To determine from one Lugol's fixed sample the relative abundance of major algal groups and to identify nondiatom algae to the genus level, we used a Wild M40 inverted plankton microscope. From the other sample permanent quantitative Hyrax mounts of diatoms were made after nitric acid treatment (Stockner 1971). Slide counts were converted to number of diatoms to per square metre (see Stockner and Armstrong 1971). The relative abundance of major algal groups calculated from inverted microscope counts was applied to this value to give total algal cells per square metre. Algal volumes were determined with values calculated by Evans and Stockner (1972) and Stockner and Shortreed (1976), although some direct volume estimates were made by equating diatoms to known geometric shapes.

The third subsample was filtered onto a 5.5-cm Whatman glass-fiber filter for determination of chlorophyll *a*. One millilitre of saturated $MgCO_3$ solution was added to prevent acidification if analysis was to be delayed. Filters were folded, placed in labeled aluminum weighing dishes, and frozen. Chlorophyll *a* was extracted by the methods of Yentsch and Menzel (1963), with minor modifications used when algal growth was exceptionally heavy. Filters were macerated in a tissue grinder with 10 mL of 90% acetone, and the filtrate analyzed for chlorophyll *a* on a Cary Model 15 recording spectrophotometer. The equation of Strickland and Parsons (1972) was used to calculate the amount of chlorophyll *a* per square metre.

The last subsample was filtered onto an ashed and weighed Whatman glass-fiber filter, folded, placed in labeled aluminum weighing dishes, and frozen. Samples were dried at 105°C to constant weight, weighed, and ashed at 500°C for 4 h in a muffle furnace, and weighed again. Values were expressed as ash-free dry weight (loss on ignition) per square metre. Net production was estimated by dividing total organic accumulation over a given sampling interval by the number of days in the interval and was expressed as milligrams C per square metre per day, where carbon was assumed to be 50% of ash-free dry weight (Stockner and Armstrong 1971).

Water samples were taken from each trough weekly, frozen, and shipped to the Pacific Biological Station, Nanaimo, B.C., for determination of nitrate and phosphate concentrations by the methods outlined in Strickland and Parsons (1972).

Stream water temperature was monitored at the intake of the pipe leading to the header box (B weir), using a Lambrecht distance thermograph (Model 256). Periodic checks of the water temperature in the troughs showed little temperature variation between troughs and B weir.

Light to the forest canopy (overstory) and the troughs beneath the canopy was recorded on a Belfort recording pyrheliometer. Total light (gram-calories per square centimetre) reaching the troughs was calculated for each sampling interval. Current velocity in the troughs was measured by the floating-chip method.

Results

In the following sections we refer to the period June 25–August 20, when nitrate and phosphate (alone and

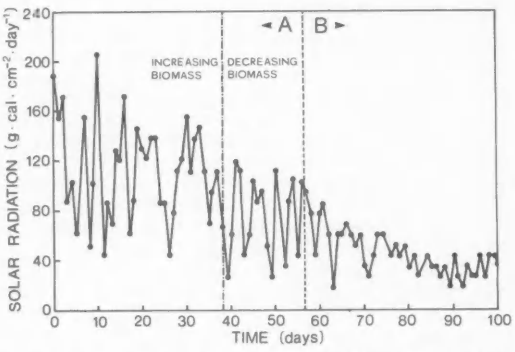

FIG. 2. Changes in daily solar radiation to streamside experiment.

combined) were added to troughs 2–4, as part A. The latter part of the experiment (August 20–October 4), when EDTA was added to nitrate and phosphate on troughs 2 and 3, and trough 4 was treated as a control to observe recovery, constitutes part B.

PHYSICAL–CHEMICAL VARIABLES

Mean daily solar radiation reaching the troughs was 98 g-cal · cm^{-2} in part A of the experiment and 47 in part B (Fig. 2). From day 0 to 38, when greatest algal growth occurred, mean daily solar radiation was 112 g-cal · cm^{-2}. In the period of rapid decline in algal abundance, day 38–59, the mean value was 74, and for the remainder of the experiment, day 59–101, the value was considerably lower, 42 g-cal · cm^{-2}.

Mean daily water temperature was constant throughout the experiment, averaging 10.1°C in part A and 10.3°C in part B of the experiment. Current velocity was 40 cm · s^{-1} in all troughs. Volume of flow in each trough was 8.4 L · min^{-1}.

Mean nitrate concentration in part A was 11 μg-atoms · L^{-1} in troughs 2 and 4 and 3.6 in troughs 1 and 3 (Fig. 3A). Mean phosphate concentration was

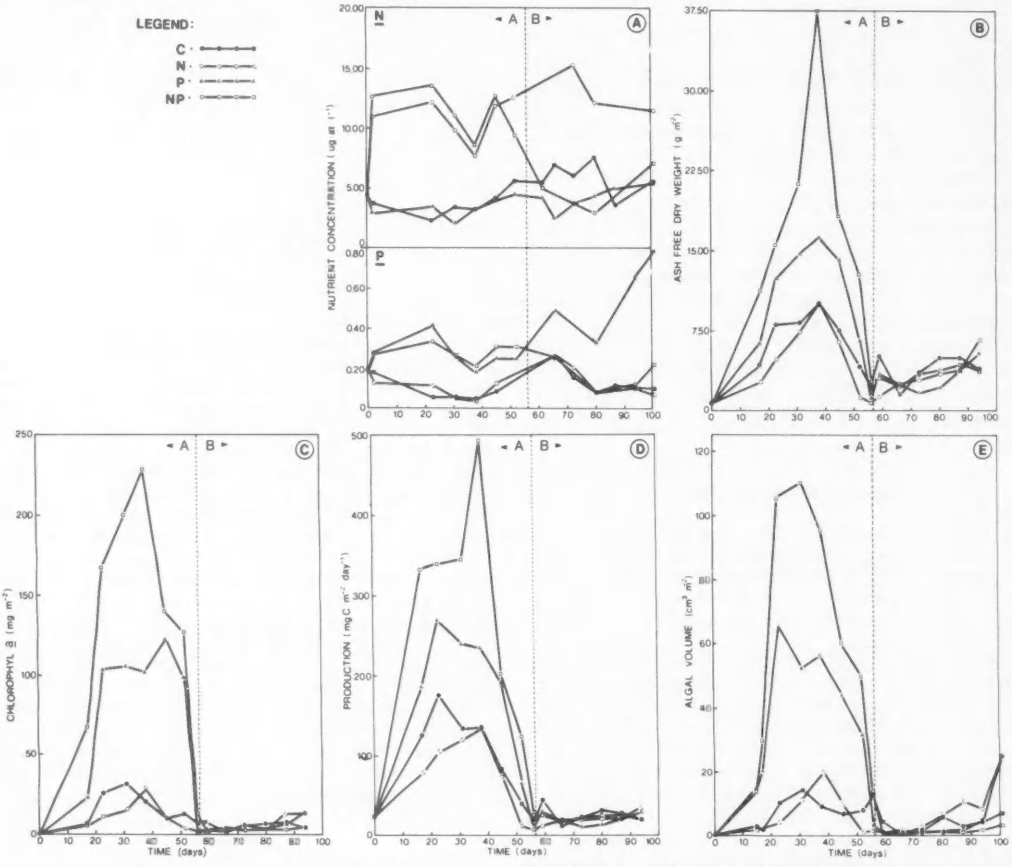

FIG. 3. Temporal variation in A, nitrate and phosphate concentrations; B, ash-free dry weight; C, chlorophyll-*a*; D, net production; and E, algal volume.

TABLE 1. Means of salient chemical and biological variables in nutrient enrichment experiment and at Station 1C in Carnation Creek, 1976. A = part A (day 0–57); B = part B (day 57–101). 1C = Station 1C, in stream 40 m below site of enrichment experiment.

		Algal vol ($cm^3 \cdot m^{-2}$)	Algal no. ($\times 10^{10} \cdot m^{-2}$)	% vol diatoms	Chloro-phyll *a* ($mg \cdot m^{-2}$)	Ash-free dry wt ($g \cdot m^{-2}$)	Nutrients ($\mu g\text{-at} \cdot L^{-1}$) NO_3	Nutrients ($\mu g\text{-at} \cdot L^{-1}$) PO_4	N:P ratio	Production ($mg\ C \cdot m^{-2} \cdot day^{-1}$)
Trough 1	A	8.0	2.71	71	17.0	6.34	3.85	0.11	35	102
(control)	B	3.8	2.04	91	6.1	3.99	5.89	0.14	42	27
Trough 2	A	8.4	1.61	74	11.3	4.81	10.85	0.09	121	75
(N)	B	7.9	2.13	94	6.6	3.47	12.93	0.13	99	22
Trough 3	A	43.2	11.55	69	80.0	10.90	3.40	0.27	13	171
(P)	B	4.9	3.98	92	6.1	3.12	4.19	0.57	7	20
Trough 4	A	78.4	20.83	71	133.6	17.06	11.37	0.29	39	265
(N & P)	B	1.7	1.48	95	3.6	3.41	5.05	0.18	28	22
1C	A	3.7	2.18	25	6.6	4.42	—	—	36	19
	B	1.5	0.29	98	0.9	0.82	—	—	36	2

0.3 μg-atoms · L^{-1} in troughs 3 and 4, and 0.10 in troughs 1 and 2. In part B, mean nitrate concentration was 13 μg-atoms · L^{-1} in trough 2, 2.5 times greater than that in the others. Phosphate concentration was increased to 0.60 in trough 3, about 4 times greater than that in the other troughs. Concentrations of EDTA were not analyzed but, by amounts mixed with phosphate and nitrate in the carboys, were estimated to be 5–10 μg-atoms · L^{-1} in troughs 2 and 3. The N:P ratio in trough 4 (30–40:1) was similar to that in Carnation Creek water (control), but the ratio ranged from 100 to 120:1 in trough 2 and from 7 to 13:1 in trough 3. Mean ambient nutrient concentrations in Carnation Creek during the course of the experiment were 3.6 μg-atoms · L^{-1} (NO_3–N) and 0.10 μg-atoms · L^{-1} (PO_4–P) with a background N:P ratio of 36:1.

BIOLOGICAL VARIABLES

Biological activity was at low levels at the beginning of the experiment (after a 4-wk colonization period). After commencement of nutrient additions, increases in algal standing stock were noted in all troughs, but most strikingly in troughs 3 and 4, receiving additions of phosphorus and nitrogen–phosphorus. Maximum periphyton accumulation was reached between days 30 and 40 (Fig. 3B–E). A rapid decline followed this initial growth period with the low point reached between days 60 and 70.

Biological activity remained low for the remainder of the experiment, although troughs 2 and 3 appeared to be increasing slowly when the experiment had to be terminated because of freshet conditions (days 90–101).

During part A of the experiment, mean values of most biological factors were 7–8 times greater in trough 4 (nitrogen–phosphorus) than in troughs 1 or 2 (control and nitrogen), and up to 5 times greater in trough 3 (phosphorus) than in 1 or 2 (Table 1). There was little difference between troughs 1 and 2.

Enrichment with nitrate and phosphate in part A did not significantly alter the relative abundance of diatoms and other major algal groups on the troughs (Table 1, Fig. 4). Filamentous green algae were present in all troughs, but in considerably greater numbers in troughs 3 and 4, with *Draparnaldia* sp., *Mougeotia* spp., *Zygnema* spp., and *Ulothrix* sp. dominant. The rhodophyte *Batrachospermum* sp. was also present but never dominant. Diatoms made up about 70% of the total algal volume in all troughs in part A and increased to over 90% dominance in part B of the experiment. The cyanophyte *Oscillatoria* sp. was present on troughs 3 and 4 but only in low numbers.

Major diatoms were *Achnanthes minutissima*, *Fragilaria vaucheriae*, *Diatoma hiemale*, and *Synedra ulna*. At the beginning of the experiment, *A. minutissima* and *F. vaucheriae* were in approximately equal numbers and constituted over 65% of the total diatom population (Fig. 5). Following nutrient addition there was a gradual increase in the relative abundance of *A. minutissima* in troughs 1, 2, and 4, amounting to over 80% of the total numbers by day 70 in these troughs. In trough 3 (phosphorus), *A. minutissima* decreased to $< 10\%$ of the total by day 20 and *F. vaucheriae* and *D. hiemale* were dominant until day 55, when they rapidly decreased. *Achnanthes minutissima* increased again to 80% by day 60. At no time did any other diatom species exceed 20% of the total diatom population, but *S. ulna*, *Tabellaria flocculosa*, *Cymbella turgida*, and *Gomphonema olivaceum* exceeded 10% at various times.

Discussion

Based on findings of a less extensive enrichment experiment, Stockner and Shortreed (1976) concluded that low-dissolved nutrients were limiting algal growth in Carnation Creek. They postulated that phosphorus was the limiting nutrient but had no direct experimental evidence. Although light was at low levels beneath the forest canopy, it was not limiting in most situations, but was considered to be the limiting factor when nutrients were abundant. Our 1976 experiments

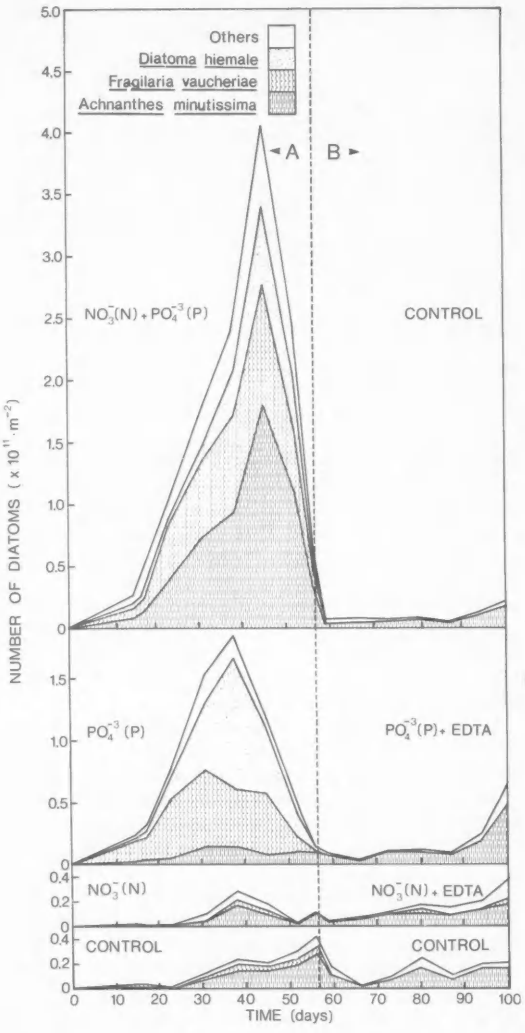

FIG. 4. Changes in the numbers of dominant diatom species on control and enriched troughs.

have demonstrated that dissolved phosphorus is the primary nutrient limiting algal growth in Carnation Creek. When the N:P ratio in trough 4 was maintained at stream levels (35–40:1), but concentrations of nitrogen and phosphorus were trebled, greatest algal growth occurred (Table 1, Fig. 3). In contrast, when phosphorus alone was trebled, and the N:P ratio was decreased to 10–13:1, maximum growth was only a little better than half that seen in trough 4. Clearly, as Schindler (1975) has demonstrated in lakes and our results have shown for streams, the N:P ratio is as important as specific nutrient concentrations in the regulation of benthic algal growth and species succession during the enhancement phase of enrichment experiments. Reduction and/or alteration of the N:P ratio from 35–40:1 to 13:1 led to a change from an *A. minutissima* dominated assemblage to one dominated by *F. vaucheriae* and *D. hiemale*. A further reduction of the ratio to as low as 5:1 would likely have led to a greater predominance of stream-dwelling nitrogen-fixing blue-greens, as it did in lakes enriched at this low ratio (Schindler 1975).

The role of incident light in regulating algal growth in Carnation Creek can be assessed by comparing these results with those of our 1975 experiments. During the trough enrichment experiments conducted in August and September 1975, good growth response was noted at average daily intensities of 80–90 g-cal • cm^{-2}. The 1976 experiment was conducted over a span of weather conditions that gave average daily conditions of 112 g-cal • cm^{-2} during the initial period of maximum growth, 74 during the period of rapid decline, and 42 during the remainder of the experiment. Lack of growth on any of the troughs during part B of the experiment was possibly due to average light intensities being be-

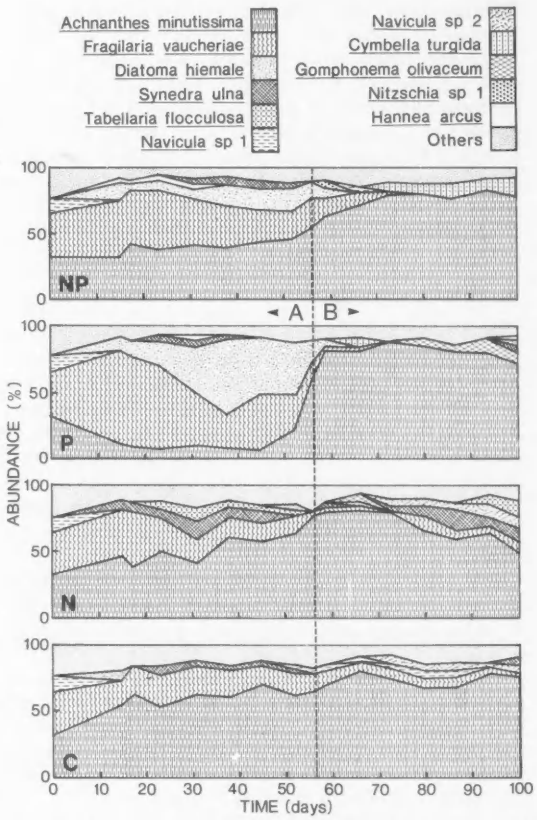

FIG. 5. Succession of major diatom species in terms of relative abundance.

low some necessary threshold. Periphyton on samplers within the stream showed a similar lack of growth during part B of the experiment (Table 1). It is likely that 50–60 g-cal · cm^{-2} · day^{-1} are minimal light requirements for benthic algal growth in streams at temperate latitudes. Riley (1967) in coastal marine waters and Lund (1949, 1954) in temperate lakes present evidence of near 40–50 g-cal · cm^{-2} threshold light-intensity requirements prior to phytoplankton bloom inception, providing other conditions are optimal.

Since temperature varied little between years of experiments or among experiments in given years, it is unlikely that it plays an important role in regulating algal growth in Carnation Creek.

The sloughing of large quantities of accumulated biomass during the period of maximum abundance during part A of the experiment can be attributed to space limitation and self-shading phenomena. Basal cells, which hold the growing mat to the substrate, cannot continue to support the continual increase in overlying biomass and these cells weaken or die because of either poor light or intolerable chemical conditions. The process of algal mat development and sloughing has been described by Stockner (1968) and Naiman (1976) in hot-spring outflow streams and by Blum (1956, 1963) in eastern streams. The density of grazing invertebrates on the troughs was so low that grazing per se could not account for the losses after 40–45 days.

Subject to the proviso that results from our streamside enrichment experiments may be validly extrapolated to other coastal oligotrophic streams in the Pacific Northwest, nutrient additions to streams while maintaining ambient N:P ratios will enhance autotrophic production. Higher trophic levels with commercial and recreational value, notably salmonids, may also benefit from enhancement. Previous studies on feeding by benthic invertebrates in Carnation Creek have shown that during periods of maximum periphyton abundance (June–October), epilithic diatoms are heavily utilized (J. C. Scrivener and K. R. S. Shortreed unpublished data). Enhancement of algal growth by controlled nutrient addition in 1976 did not alter species succession from diatoms toward filamentous blue-green or green algal assemblages, which are less useful to grazers. Thus, enhancement of autotrophic production in coastal streams may increase the carrying capacity of secondary and tertiary trophic levels, providing food during summer and fall periods is the limiting factor. The validity of these assumptions could be tested in Carnation Creek, where currently most coho salmon (*Oncorhynchus kisutch*) fry require 2 yr of freshwater rearing prior to smoltification and seaward migration instead of the normal 1-yr residence (Narver and Andersen 1974).

Following logging of the Carnation Creek watershed and removal of the streamside vegetation, algal production will likely increase rapidly because of increased nutrient income and improved light conditions. However, data from Ritherdon Creek on an adjacent clearcut logged (1968–69) watershed show that restoration of oligotrophic conditions despite excellent light conditions will occur within 3–4 yr because of reduced nutrient levels (Stockner and Shortreed 1976). If we compare our results with those of McIntire and Phinney (1965) from laboratory streams, our maximum net production on trough 4 should have been 4–5 times higher than our observed mean value at peak production, because our nutrient levels were as high as theirs. It failed to reach these high levels because of low light at the streamside location of our experiments. Thus, enrichment of coastal streams after logging would be one way of sustaining maximum periphyton production with possible benefits to higher trophic levels. As in lakes, too much enrichment or serious alteration of the ambient N:P ratio could result in algal assemblages dominated by green or blue-green algae, which would be detrimental to invertebrates and fish populations if sustained throughout the growing season. Therefore, before stream enhancement by fertilization can become an operational management technique, it is essential to conduct further experiments similar in scope to these here described to elucidate species succession and growth response to a variety of nutrient concentrations and N:P ratios.

Acknowledgments

We acknowledge the support of this study by the Carnation Creek Coordinating Committee and the Pacific Biological Station, Nanaimo. Many persons gave generously of their time in aspects of this work. Messrs B. Andersen, T. Brown, M. Lambert and T. Nason assisted in field operations. Mr T. Nason also provided the necessary chemical analyses. Mr C. Scrivener helped coordinate the program and with Dr P. E. K. Symons provided a critical review of this manuscript.

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Toxicity of Cadmium to Goldfish, *Carassius auratus*, in Hard and Soft Water[1]

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McCarty, L. S., J. A. C. Henry, and A. H. Houston. 1978. Toxicity of cadmium to goldfish, *Carassius auratus*, in hard and soft water. J. Fish. Res. Board Can. 35: 35–42.

Variations in cadmium form and concentration and in selected water quality parameters (pH, dissolved oxygen, total hardness, total alkalinity, conductivity) were monitored during static bioassays conducted with relatively soft (~ 20 mg/L as $CaCO_3$) and hard (~ 140 mg/L as $CaCO_3$) waters. Cadmium concentrations were reasonably stable in soft water, and with the exception of total hardness, water quality was not greatly altered during assay. Cumulative mortality curves were of a simple sigmoidal type and readily analyzed by conventional procedures. LC50 values of 2.76, 2.13, and 1.78 mg Cd/L were estimated on the basis of 48-, 96-, and 240-h periods of observation. During hard-water trials there were transient increases in the amount of particulate cadmium present and sharp decreases in total cadmium levels. Several parameters (pH, total alkalinity, conductivity) exhibited transient and/or sustained variations of a cadmium concentration-dependent type. Mortality curves were typically biphasic. The extent of first-phase mortality was significantly correlated with the magnitude of the initial pH decline and the amount of cadmium present in centrifugable form. Conventional procedures did not result in rectification of 240-h cumulative mortality curves, and the 240-h LC50 value (40.2 mg Cd/L) is considered to be inherently less precise than those obtained on the basis of 48- and 96-h periods of observation (46.9, 46.8 mg Cd/L).

Key words: goldfish, *Carassius auratus*, cadmium toxicity, hard water, soft water, mortality

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Des essais biologiques statiques effectués dans des eaux relativement douces (~ 20 mg/L comme $CaCO_3$) et dures (~ 140 mg/L comme $CaCO_3$) ont servi à suivre les variations de forme et de concentration du cadmium et de quelques paramètres de la qualité de l'eau (pH, oxygène dissous, dureté totale, alcalinité totale, conductivité). Les teneurs en cadmium sont raisonnablement stables en eau douce et, sauf pour ce qui est de la dureté totale, il n'y a que peu de changement dans la qualité de l'eau au cours de l'essai. Les courbes de mortalité cumulative sont du type sigmoïde simple et se prêtent facilement à l'analyse par des méthodes conventionnelles. Les valeurs de CL50 de 2.76, 2.13 et 1.78 mg Cd/L ont été estimées pour des périodes de 48, 96 et 240 h. Au cours des essais en eau dure, nous avons observé des augmentations passagères de la quantité de cadmium particulaire présent et de brusques diminutions des niveaux de cadmium total. Plusieurs paramètres (pH, alcalinité totale, conductivité) accusent des variations passagères et/ou soutenues de type dépandant de la teneur en cadmium. Les courbes de mortalité ont typiquement deux phases. L'étendue de la mortalité durant la première phase montre une corrélation significative à la fois avec l'étendue du déclin initial du pH et avec la quantité de cadmium présent sous une forme propice à la centrifugation. Les méthodes conventionnelles sont impuissantes à rectifier les courbes de mortalité cumulative après 240 h, et la valeur de la CL50 après 240 h (40.2 mg Cd/L) est considérée comme moins précise que les valeurs obtenues après des périodes d'observation de 48 et de 96 h (46.9, 46.8 mg Cd/L).

Received July 27, 1976
Accepted September 1, 1977

Reçu le 27 juillet 1976
Accepté le 1 septembre 1977

Depending upon water hardness, particularly in closed systems, substantial changes in test conditions are known to occur during cadmium bioassay (Pickering and Henderson 1966; Pickering and Gast 1972). These are, in part at least, qualitatively predictable on the basis of known cadmium reactions in aqueous solution (Davies 1976). Hartung (1973) has, however, noted the lack of information concerning the time course of such changes

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Printed in Canada (J4486)
Imprimé au Canada (J4486)

during cadmium and other heavy metal toxicity assays, and the influence, if any, which they may have upon mortality patterns and consequently upon estimated LC50 values is uncertain. Since accurate assessment of acute toxicity is an essential step in the development of realistic water quality criteria and since much of the data pertinent to heavy metals has been derived from static bioassay studies, the question is one of practical concern. The present investigation was undertaken to ascertain whether mortality patterns in a recommended test species, the goldfish, *Carassius auratus*, could be related to changes taking place during assays carried out in relatively soft and hard water.

Materials and Methods

General

Two series of trials were carried out. The first involved evaluation of changes within the test system during simulated 96-h assays. Four initial concentrations (nominally 1, 10, 20, and 50 mg Cd/L) were used in conjunction with two levels of water hardness (initially 20 and 140 mg/L as $CaCO_3$), and samples were drawn for analyses at 0, 0.5, 1, 4, 8, 16, 24, 48, 72, and 96 h. Total hardness and alkalinity, pH, and dissolved O_2 were determined at all intervals other than 30 and 60 min.

The 240-h bioassays were subsequently carried out using procedures recommended by Sprague (1973). Initial nominal concentrations were as follows: in soft water (20–22 mg/L as $CaCO_3$) — 1, 2, 3, 5, and 10 mg Cd/L; in hard water (143–145 mg/L as $CaCO_3$) — 5, 10, 20, 30, 35, 40, 45, 50, and 60 mg Cd/L. Samples were taken for cadmium analysis at 0, 6, 12, 24, 48, 72, 96, 120, 144, 168, 192, 216, and 240 h. Temperature, dissolved O_2, pH, and conductivity were determined at these intervals and also at 15, 30, 60, 120, and 240 min. Total hardness and alkalinity were measured only at the commencement and termination of each trial in this series.

Solution Preparation and Analytical Methods

Well-aerated, dechlorinated, and aged St. Catharines city water ($Cd < 10\ \mu g/L$) was used throughout the study; media of reduced hardness were prepared by dilution with glass-distilled water. All tests were carried out in 100-L volumes of freshly prepared solution in fiberglass aquaria. The latter were equipped with laterally positioned, submerged air-bars for aeration and mixing, and translucent plastic hoods for minimization of evaporative loss and visual disturbance of specimens. Photoperiod was held at 14hL:10hD during trials. Over the course of the study temperatures ranged from 22.0 to 25.4°C. Maximum variation during any given trial was, however, less than this (1.5°C).

Aquaria were washed with Contrad-70 (Harleco) and thoroughly rinsed to reduce cadmium background. Appropriate amounts of analytical grade $CdCl_2$ (BDH) were dissolved in small volumes of water and mixed into each tank to establish initial levels. Fixed siphons were used to withdraw samples from the center of each aquarium, and cadmium determinations were carried out with a Unicam SP-90 AAS used in the absorption mode. Addition of cadmium to hard waters leads to precipitation of carbonate and hydroxide complexes, and it was considered desirable to evaluate the relationship between cadmium present in solution or as a finely divided suspension (i.e. that which can be taken up by diffusion or phagocytosis) and that occurring in particulate form and therefore less likely to be rapidly absorbed. The filtration procedures ordinarily used for this purpose are known to induce error through adsorption (John and Van Laerhoven 1976). Consequently, aliquots of each sample were centrifuged ($10{,}000 \times G$, 30 min), and subsamples drawn from the upper portion of the supernatant for reanalysis. Differences between the concentrations of raw and centrifuged samples were then taken as being at least indicative of the relative amounts of dissolved plus finely divided, and particulate cadmium present.

Total hardness and alkalinity were determined titrimetrically (APHA et al. 1971). Dissolved oxygen was estimated with a Yellow Springs Instrument 51A oxygen meter calibrated by the PAO modification of the Winkler method. A Fisher Accumet 140 meter was used for pH measurements, and a Chemtrix 70 conductivity meter for conductivity determinations.

Experimental Animals

Goldfish (1.93 ± 0.73 g) were held at temperatures similar to those at which tests were conducted for 2 wk before use, and fed twice daily ad lib. No prophylactic treatments were employed, as stocks were judged healthy on the basis of general activity, feeding behavior, and absence of overt disease symptoms.

Feeding was terminated 2 days prior to transfer to test tanks, and a 3-day interval allowed for recovery from handling stress. Specimens were not fed during this and the subsequent trial period. Fecal matter was regularly removed during the recovery period to reduce possible cadmium depletion by adsorption. Trials were replicated to provide a total of 50 animals at all test concentrations other than 5, 10, and 20 mg Cd/L (hard water). No mortalities were encountered in the latter trials, and tests were therefore terminated with samples of 30 specimens. Each tank was examined at 1, 2, 4, and 8 h, and thereafter at 8-h intervals. Animals were considered to be dead when they were immobile, showed no respiratory activity, and failed to respond to probing of the caudal peduncle. Lengths and weights were recorded, and selected specimens examined with particular emphasis upon gill and gut tract appearance.

Data Evaluation

Statistical analyses were carried out as described by Sokal and Rholf (1969), and mortality data evaluated using the procedures outlined by Sprague (1973).

Results

Simulated 96-h Assays

Cadmium — Mean initial cadmium levels in soft water were 0.9, 8.5, 16.5, and 48.0 mg Cd/L; in hard water 0.9, 8.5, 18.0, and 49.8 mg Cd/L. Total cadmium fell sharply in hard water, declining to 10% or less of initial concentration within 96 h (Fig. 1). Much of the cadmium present during the early phases of observation was removable by centrifugation, presumably in the form of relatively large particles. By contrast, total cadmium was generally stable in soft water, and only

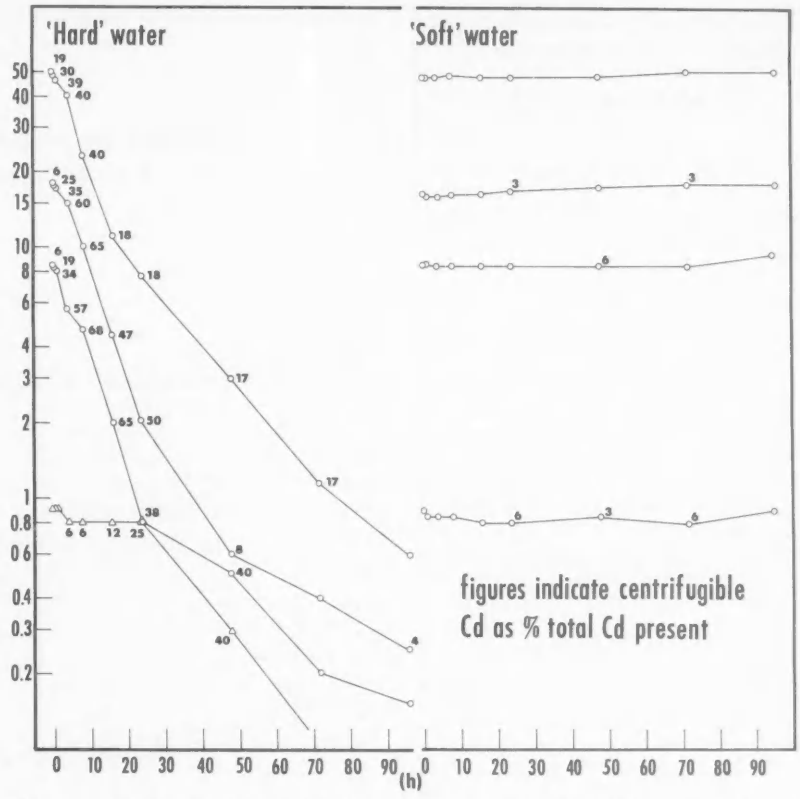

FIG. 1. Variation in total cadmium (mg Cd/L) during simulated 96-h assays conducted with soft (20 mg/L as $CaCO_3$) and hard (140 mg/L as $CaCO_3$) water media.

negligible amounts were occasionally present in centrifugable form.

WATER QUALITY

Dissolved O_2 remained reasonably steady at values exceeding 90% saturation, and well above the recommended minimum of 4 mg/L. This was also true of total alkalinity (soft water) which fluctuated between 14 and 18 mg/L (as $CaCO_3$) with no evidence of any consistent trend. These data have accordingly been omitted from the summary of water quality characteristics depicted in Fig. 2. Although total hardness increased immediately upon addition of cadmium to soft water, this was not the case in hard water. Only in the instance of the nominal 50 mg Cd/L trials was a significant change in hardness seen, and this was transient in nature. Modest increases in the pH of control and nominal 1 mg Cd/L soft water tanks were encountered. At higher cadmium concentrations, however, pH decreased transiently. More substantial decreases in pH occurred in hard water and were accompanied by reductions in total alkalinity. It will be apparent that the magnitudes of these variations were related to initial cadmium concentrations.

240-h BIOASSAYS

Cadmium — There was little change in cadmium levels in soft water (1.1, 1.2, 3.2, 5.1, and 10.2 mg Cd/L) and again the differences between centrifuged and uncentrifuged samples were negligible. Marked decreases in total cadmium were observed in hard-water trials. That not removable by centrifugation declined by 50–75% within 6 h and in general was reduced to <5% of the original concentration over the period 72–240 h (Fig. 3). Centrifugable cadmium, on the other hand, increased sharply during the initial 6-h period, accounting for 30–50% of the amount originally present.

WATER QUALITY

Variations in water quality characteristics during 240-h bioassays were similar to those described for the 96-h trials (Fig. 4). Oxygen fluctuated around the 8 mg/L level. Hardness increased in soft water, but

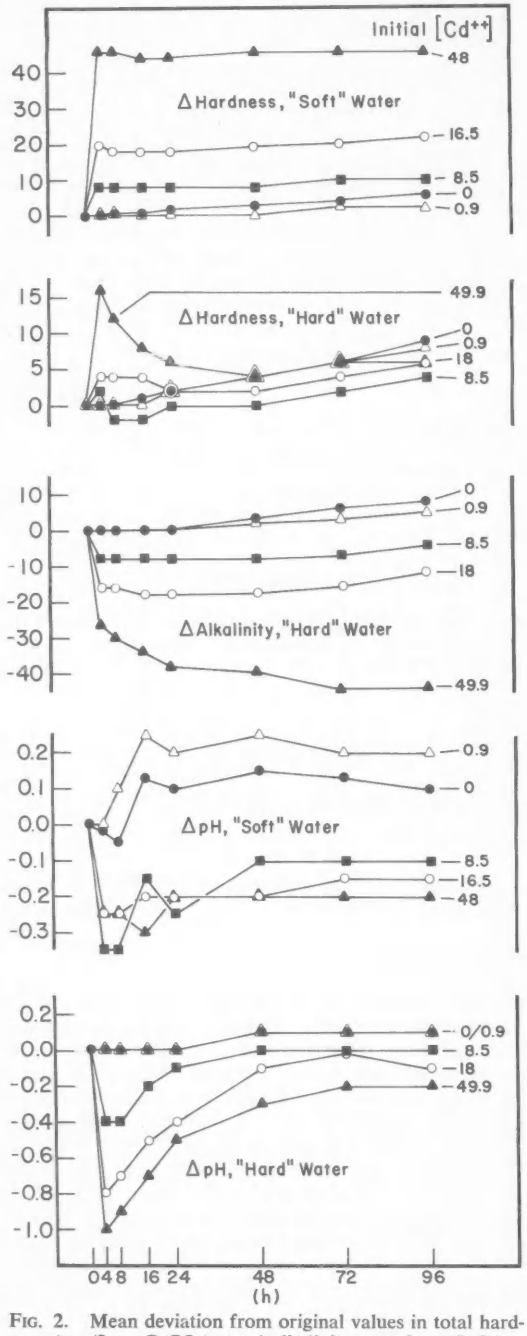

FIG. 2. Mean deviation from original values in total hardness (mg/L as $CaCO_3$), total alkalinity (mg/L as $CaCO_3$), and pH during simulated 96-h assays conducted with soft (20 mg/L as $CaCO_3$) and hard (140 mg/L as $CaCO_3$) water media.

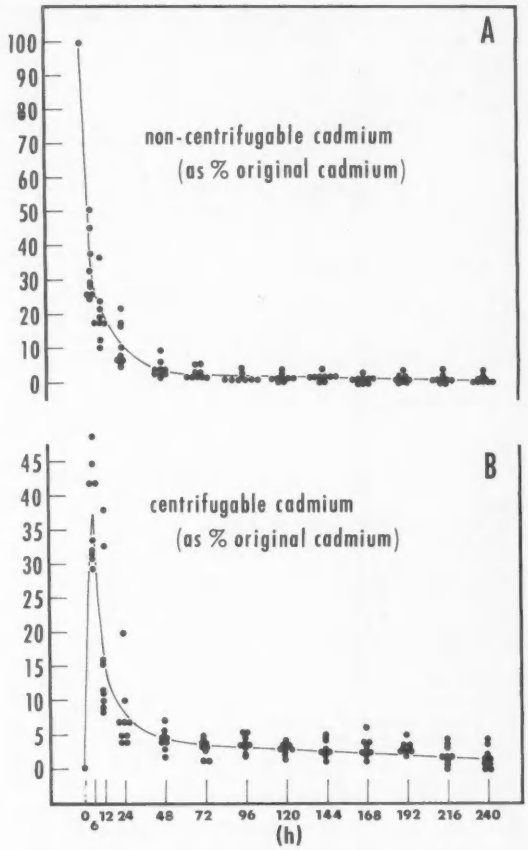

FIG. 3. Variations in noncentrifugable and centrifugable cadmium levels, as percentages of initial cadmium content, during 240-h bioassays conducted in hard water (143–145 mg/L as $CaCO_3$).

varied little in hard water. Total alkalinity declined in hard water, with only minor changes taking place under soft-water conditions. Transient increases in hydrogen ion concentration were observed in both test media. Conductivity variations tended to parallel those previously noted for hardness. Cadmium addition to soft water resulted in an immediate increase in the conductivity of solutions relatively rich in cadmium, but little variation thereafter. By contrast, the conductivity of more dilute soft-water solutions increased steadily with time. Marked, but transient, increases in conductivity were followed by reductions to reasonably steady levels within 24–48 h in hard-water trials. Again, deviations in water quality were clearly related to original cadmium content.

MORTALITY PATTERNS

Of the 100 specimens used in the soft- and hard-water control series only one died (96 h, soft water). Autopsy

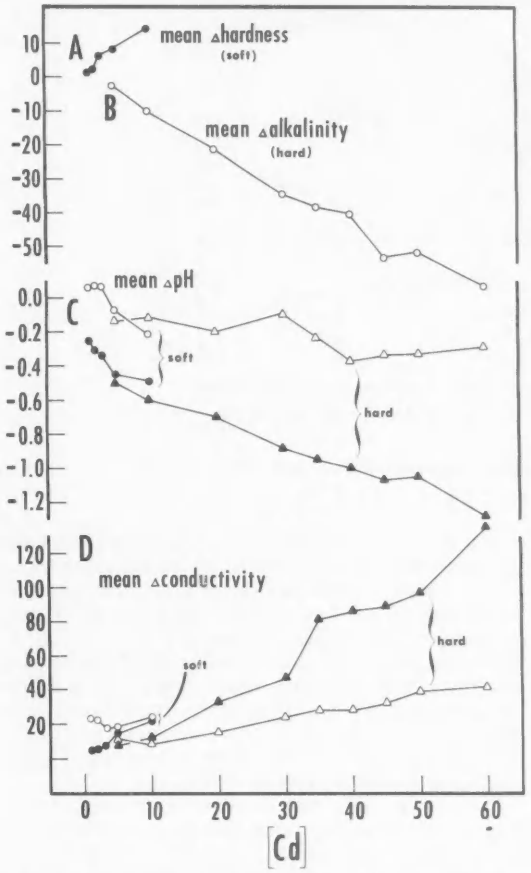

FIG. 4. Mean deviation from original values in (A) total hardness (mg/L as $CaCO_3$) in soft water (20–22 mg/L as $CaCO_3$), (B) total alkalinity (mg/L as $CaCO_3$) in hard water (143–145 mg/L as $CaCO_3$), (C) pH, and (D) conductivity (micromhos/cm) in hard and soft water during 240-h bioassays as functions of initial total cadmium level. Solid symbols: maximum initial deviation from 0-h values; open symbols: final stable deviation from 0-h values.

revealed no obvious cause of death.

Mortality was complete within 16 and 24 h after exposure to 5 and 10 mg Cd/L solutions in soft water (Fig. 5). At all lower concentrations mortality was incomplete; plateaux of 88% (3 mg Cd/L), 60% (2 mg Cd/L), and 16% (1 mg Cd/L) being reached after 136, 216, and 184 h, respectively. Estimates of 48-, 96-, and 240-h LC50 values (2.76, 2.13, 1.78 mg Cd/L) were in good agreement with those previously reported for this species on the basis of assays conducted with water of comparable hardness (Pickering and Henderson 1966).

A more complex situation was encountered in hard-water trials (Fig. 6). Only the highest (60 mg Cd/L) and lowest (30 mg Cd/L) effective test concentrations lead to the simple sigmoidal cumulative mortality curves seen in soft-water tests. At all intermediate concentrations mortality plateaux were reached after 30–80 h and persisted for 60–90 h. A second phase of rapid mortality then ensued. This occasioned only a modest increase in overall mortality at 50 mg Cd/L, but accounted for progressively larger proportions of the total in the less-concentrated test solutions.

Significant ($P < 0.01$) correlations were found between the extent of first-phase mortality and both the magnitude of initial pH decrease ($r = 0.938$) and maximum centrifugable cadmium concentration ($r = 0.884$). Similarly, mortality was correlated with final pH and decrease in total alkalinity. In neither phase of mortality were correlations between weight and resistance time observed. However, the weight range of the test population was quite restricted (0.82–4.15 g).

Symptomatic differences were also associated with the two phases of hard-water mortality. With the onset of the initial phase, specimens frequently surfaced and "gulped" air; a behavior pattern associated with massive branchial mucous accumulation. In this, and in mucous strands extending from the lateral surfaces and anus, fine particulate material was clearly visible. Mucous accumulation was also observed within the gut tract, as was evidence of general hemorrhagic deterioration. The subjective impression was gained that extent of branchial mucous deposition and particle density in mucus were related to initial cadmium concentration. Particulate cadmium, acting as a surface irritant, may have prompted mucous secretion. These symptoms were less evident during the latter part of first-phase mortality (survivors apparently sloughing much of the material off) and totally absent during the succeeding period of rapid mortality.

On the basis of 48-, 96-, and 240-h exposures, LC50 values of 46.9, 46.8, and 40.2 mg Cd/L were estimated. The first of these include only first-phase mortality, that for 240 h, both phases. However, the biphasic mortality curves associated with exposure to cadmium levels of 35–50 mg/L were not rectifiable by means of the transformations normally used to linearize mortality data. Accordingly, the 240-h LC50 estimate of 40.2 mg Cd/L must be regarded as more approximate in character than those based upon 48- and 96-h observation periods.

Discussion

The dose–response approach to assessment of pollutant toxicity embodies several assumptions. Unless these are satisfied under actual test conditions the inferences that may be drawn from the resulting mortality data are questionable in any rigorous sense, although they may, of course, provide useful pragmatic information. Three appear to be particularly critical: that test organism sensitivity to the toxicant is normally distributed or transformable to an acceptable approximation of normality, that the toxicant challenge confronting the test organism is quantitatively and qualitatively stable with

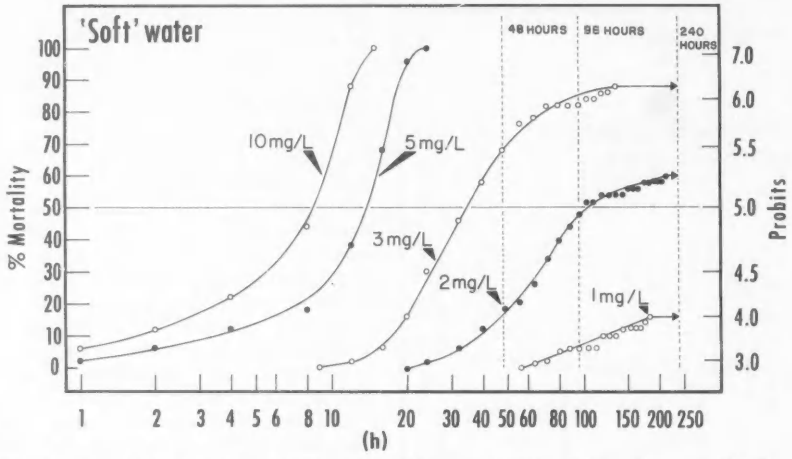

FIG. 5. Cumulative mortality during 240-h bioassays conducted in soft water (20–22 mg/L as $CaCO_3$).

respect to time, and that the mechanism(s) causing death are qualitatively independent of dosage and other trial conditions. Given these circumstances mortality curves can be readily rectified, and medium resistance times and LC50 values estimated with some confidence.

Our data clearly suggest that for all practical purposes, these assumptions were satisfied in trials carried out in relatively soft water. It is equally clear that cadmium bioassay under hard-water conditions is associated with variations in the amount and form of toxicant and in test conditions. The correlations obtained between several of the latter and mortality are at least suggestive of cause–effect relationships. It is appropriate, therefore, to consider the possible basis of these changes, and their potential effects upon test organisms.

Heavy metal reactions in solution, including those of cadmium (Gardiner 1974; Davies 1976), have been extensively investigated. Cadmium is notable for a tendency to complex with carbonate and hydroxide, and it is of interest that the reactions

$$Cd^{2+} + 2H_2O \rightarrow Cd(OH)_2 \downarrow 2H^+$$

$$Cd^{2+} + 2HCO_3^- \rightarrow CdCO_3 \downarrow 2H^+$$

will account for many of the variations observed. Each millimole of cadmium added to the system will neutralize 2 mM of bicarbonate alkalinity, and the decreases in total alkalinity noted (Fig. 2, 3B) correspond to this. Reductions in total cadmium necessarily follow, as bicarbonate and hydroxide complexes are only sparingly soluble. Reactions of this general type will also lead to the observed increases in acidity, as would formation of $Cd(OH)^+$. Recovery of pH might then follow from reactions of the type

$$Cd(OH)_n + HCO_3^- \rightarrow CdCO_3 \downarrow + H_2O + (OH^-)_{n-1}$$

if the initial step favors the metastable hydroxide, and would lead to further depletion of cadmium from the water column by precipitation of the insoluble carbonate.

The initial increase in hydrogen ion concentration would also be expected to favor the existence of cadmium in one or more ionic forms (e.g. Cd^{2+}, $Cd(OH)^+$), which are presumably more readily absorbed than insoluble complexes.[2] Some support for this supposition can, in fact, be adduced from the recent demonstration that the initial stages of radiocadmium uptake by freshwater fishes proceed more rapidly in hard than in soft water (Kinkade and Erdman 1975).

The nature of the mortality curves obtained in soft and hard water points to the likelihood of at least two modes of cadmium lethality. A variety of syndromes have been observed in specimens exposed to effective or lethal concentrations of cadmium in moderately to extremely hard water (e.g. Gardner and Yevich 1970; Jackim et al. 1970; Bilinski and Jonas 1973; Voyer et al. 1975; McCarty and Houston 1976; Larsson et al. 1976). In many instances these must involve actual cadmium absorption and subsequent interactions at the enzyme and/or structural protein level of the kinds encountered with other metal ions (Friedberg 1974). Given earlier assumptions concerning the uptake of ionic cadmium and soluble versus sparingly soluble cadmium complexes, this facet of cadmium action may be largely confined to the early stages of exposure in hard water. Uptake at very much reduced rates consistent with the decreased availability of cadmium in this form would, however, presumably occur throughout exposure. Internal effects probably account for all or most of the action of cadmium in soft water.

The apneic behavior of test specimens prior to and during the onset of rapid first-phase mortality in hard

[2]We are grateful to one of the manuscript referees who suggested this interpretation of the data.

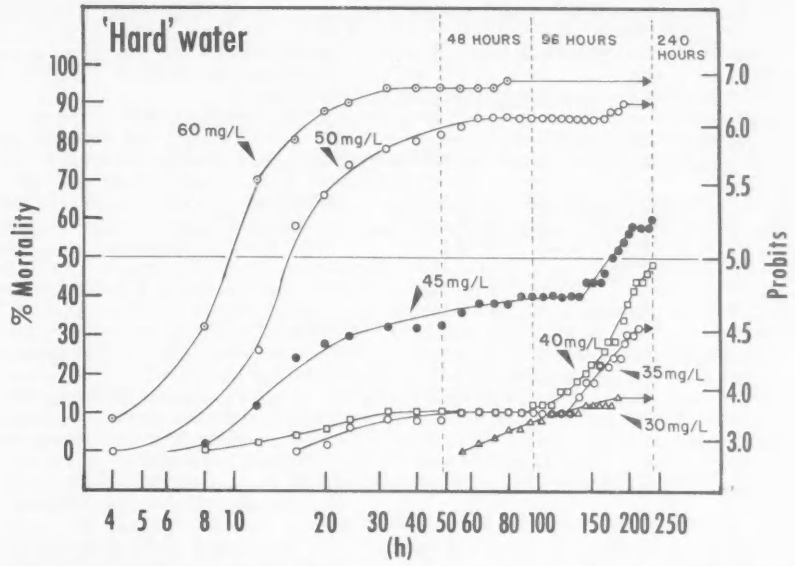

FIG. 6. Cumulative mortality during 240-h bioassays conducted in hard water (143–145 mg/L as $CaCO_3$).

water is, on the other hand, compatible with the hypothesis that cadmium reduces gas-exchange efficiency (Burton et al. 1972). The extensive formation of mucus on the gills must prompt some degree of diffusional anoxia. Furthermore, cytological evidence of lamellar epithelial cell breakdown has been reported following exposure of freshwater fishes to a variety of metallic toxicants.

Other less obvious effects may, however, be entrained by exposure to cadmium in hard water. Reductions in pH of the magnitude observed under these conditions will prompt similar changes in blood pH and these, in turn, may lead to a functional hypohemoglobinemic vascular hypoxia as a consequence of effects upon hemoglobin–oxygen affinity (Packer and Dunson 1970, 1972; Brunori 1975; Eddy 1976). Increases in environmental hydrogen ion levels are also associated with significant increases in ventilatory rate and flow (Eddy 1976; Hargis 1976). These presumably prompt cadmium concentration-dependent increases in rate of cadmium presentation to the gills and may lead to a form of positive feedback. Hyperventilation, in bringing more particulate cadmium to the lamellar surface, would, for example, presumably prompt increased mucous formation. The resulting decrease in oxygen transfer rates would then induce vascular hypoxia, stimulation of ventilatory activity, more rapid cadmium deposition, and further mucous formation. The generally exponential nature of positive feedback responses may, in fact, underlie the marked discontinuities in mortality encountered between the moderate and higher cadmium test solutions.

Finally, a further pH effect has been described by Packer and Dunson (1970, 1972). Sodium uptake in freshwater fishes involves Na^+/H^+ exchange and intervention of the active (Na^+/K^+)-stimulated ATPase system. The latter is highly sensitive to pH (Giles and Vanstone 1976). It is not surprising, therefore, that exposure to increased hydrogen ion levels is accompanied by massive sodium loss. Furthermore, there is evidence that survival under such conditions is inversely related to sodium depletion rate. Although Packer and Dunson regarded anoxia as the primary cause of death at low pH, studies involving the use of isotonic sodium chloride test media provided some indication that sodium loss itself may cause death. Thus, at higher cadmium levels some component of lethality may well stem from concommitant pH effects, and not from cadmium action per se. Indeed, as many of the transition metals undergo reactions comparable to those of cadmium (Cotton and Wilkinson 1972), static bioassay data for these may incorporate a "hidden" pH component as well.

Acknowledgments

The authors gratefully acknowledge the interest and advice of Drs M. F. Richardson and J. M. Miller of the Department of Chemistry, Brock University, and of their colleague Mr P. G. F. Murphy. Supported by internal funding to A. H. Houston.

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Clinal Variation in Scale Characters of Atlantic Salmon (*Salmo salar*) Based on Discriminant Function Analysis

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LEAR, W. H., AND R. K. MISRA. 1978. Clinal variation in scale characters of Atlantic salmon (*Salmo salar*) based on discriminant function analysis. J. Fish. Res. Board Can. 35: 43–47.

Scales of adult Atlantic salmon (*Salmo salar*) were collected from 18 river systems in eastern North America from 55°33′N in Labrador to 44°33′N in Maine and to 44°23′N in Nova Scotia during 1968, 1973–75. Analysis of four scale characters, namely circuli in first annual river zone, circuli in second annual river zone, smolt age, and circuli in first annual marine zone, demonstrated that significant differences occurred in each of these characters between river systems and latitude. The numbers of circuli in each of the three growth zones increased from north to south, while the smolt ages decreased from north to south. A discriminant function applied to these four characters employing latitude as a "dummy" variable indicated that the discriminant function contributed significantly to the clinal trend and 73.7% of the total variation was explained by this discriminant function.

Key words: adult Atlantic salmon; scale characters; salmon; latitudinal cline, scale characters; clinal discriminant function

LEAR, W. H., AND R. K. MISRA. 1978. Clinal variation in scale characters of Atlantic salmon (*Salmo salar*) based on discriminant function analysis. J. Fish. Res. Board Can. 35: 43–47.

Des écailles de saumon atlantique (*Salmo salar*) adultes ont été recueillies dans 18 réseaux fluviatiles d'Amérique du Nord orientale depuis les latitudes 55°33′N au Labrador jusqu'à 44°33′N dans le Maine et 44°23′N en Nouvelle-Ecosse en 1968 et de 1973–1975. L'analyse de quatre caractères de l'écaille, soit les circuli dans la première zone annuelle de vie en rivière, les circuli dans la seconde zone annuelle de vie en rivière, l'âge des smolts et les circuli dans la première zone annuelle de vie en mer, révèle des différences significatives, dans chacun de ces caractères, entre les réseaux fluviatiles et la latitude. Le nombre de circuli dans chacune des trois zones de croissance augmente du nord au sud, alors que l'âge des smolts diminue du nord au sud. L'application d'une fonction discriminante à ces quatre caractères, utilisant la latitude comme variable «factice», indique que la fonction discriminante contribue de façon significative à la tendance clinale et que cette fonction discriminante rend compte de 73.7% de la variation totale.

Received June 1, 1977
Accepted September 14, 1977

Reçu le 1 juin 1977
Accepté le 14 septembre 1977

THE smolt ages of Atlantic salmon (*Salmo salar*) exhibit a general average increase from south to north along the northeastern coast of North America (Templeman 1967). This is also reflected in the smolt ages of Atlantic salmon taken in the commercial fisheries in Newfoundland and Labrador (Lear and May 1972; Lear 1973). Payne (1974) reported that a latitudinal cline was indicated by the frequency of the *Tf4* transferrin allele in North American populations of Atlantic salmon. Lear (1972) found that differences in scale characteristics of Atlantic salmon from Europe and North America were of sufficient magnitude to possibly serve as a basis for discriminating between these stocks in the West Greenland fishery area. Lear and Sandeman (1974) subsequently used scale characters and a discriminant function for identifying the continental origin of Atlantic salmon in the West Greenland area. The purpose of this paper is to demonstrate that a latitudinal cline is evident in the scale characteristics of Atlantic salmon in northeastern North America and that a discriminant function analysis based on scale characters could be useful in identifying stocks of salmon.

Printed in Canada (J4859)
Imprimé au Canada (J4859)

Materials and Methods

SCALE COLLECTION AND ANALYSIS

Scales of adult Atlantic salmon were collected from rivers in Labrador, Newfoundland, New Brunswick, Nova Scotia, and Maine during 1968, 1973–75 (Fig. 1, Table 1). The samples from Newfoundland–Labrador were all from angling catches except for those from Indian River, which

FIG. 1. Atlantic salmon rivers of North America from which adult Atlantic salmon were sampled for scale collections and which form the basis of the discriminant function analysis.

were obtained at the controlled-flow spawning channel. The samples from the Miramichi, Margaree, and LaHave rivers were from sampling traps, while those from the North, St. Mary's, and Narraguagus rivers were from angling catches. Sample sizes are shown in Table 1. Since in most cases the sex of the fish was not recorded, the samples were not analyzed for sex differences.

Scales were taken from the left side of the fish approximately midway between the lateral line and the dorsal fin and generally in a line between the dorsal and anal fins. They were washed in a mild detergent to remove the pigmentation, and impressions of them were made on preheated plastic slides. These impressions were projected onto the ground-glass screen of a microprojector at a magnification of 30×. The river and sea ages were then interpreted from the scale impressions. Four characters were examined and incorporated into the discriminant function analysis. These four characters were (1) number of circuli in 1st river year (CR_1), (2) number of circuli in 2nd river year (CR_2), (3) river age at smolt migration (RA), (4) number of circuli in 1st sea year (CS_1).

The problem of counting broken or branched circuli was solved by counting only those circuli that continued intact within an angle of 10° on each side of a line drawn through the longest axis of the scale. The criteria for exclusion or inclusion of circuli for counting were similar to those used by Tanaka et al. (1969).

In the analysis, the samples from the Pinware, Hunt, Sand Hill, St. Mary's, LaHave, Miramichi, Margaree, and North rivers were separated on the basis of small (1-sea-winter) and large (2- and 3-sea-winter) salmon in an attempt to determine whether differences existed in the scale characters of salmon maturing after varying periods at sea. In addition, Student's *t*-tests were applied to the sea age-groups from the above eight rivers to determine whether a significant relationship existed between river age and sea age.

A clinal discriminant function was fitted to the data using latitude as an independent variable, where latitude (°N) is the position of the mouth of the river.

Scales of adult Atlantic salmon were collected from 125 individuals tagged in the Newfoundland–Labrador area and eventually recaptured in 47 rivers in eastern Canada from 53°34′30″N to 44°59′00″N. The clinal discriminant function derived from the basic river data was applied to the scale characters of the tagged–recaptured fish.

Analyses and Results

Wilk's lambda criterion (Kendell and Stuart 1966) was employed for a one-way multivariate ANOVA to test the null hypothesis that the vector of means of four measurements were equal for all 26 samples. The likelihood ratio test statistic estimated was: $\mu_{(4,25,746)} = 0.0866$ yielding $P < 0.01$. This indicated that the means for areas were not all equal. Student's *t*-tests performed on samples from the above eight rivers suggested that there was no significant difference in river ages between small and large salmon from the Pinware, Hunt, Sand Hill, and St. Mary's rivers. Large salmon from the LaHave, Margaree, and North rivers had significantly lower river ages than small salmon, while large salmon from the Miramichi had significantly higher river ages than small salmon. Multivariate ANOVA was done between small (1-sea-winter) and large (2- and 3-sea-winter) salmon from the Pinware, Hunt, Sand Hill, St. Mary's LaHave, Miramichi, Margaree, and North rivers. For the Pinware, Hunt, and Sand Hill rivers, there was no difference between small and large salmon ($P > 0.05$), while the small and large salmon from Miramichi, Margaree, North, and LaHave rivers were significantly different at 1% probability level and those from St. Mary's River at 5% probability level. In the analysis to follow, samples of pairs that differed significantly were considered separately, while those of pairs that did not differ significantly were pooled as single samples, thus making a total of 23 samples (instead of the original 26). The likelihood ratio test statistic $\mu_{(4,22,749)}$ estimated for these 23 samples was 0.0889 with P still <0.01. Figure 2 gives a plot of the values of the mean against latitude, where latitude (°N) was the position of the mouth of the river. This showed that the means were widely spread over the latitudes; furthermore, there was also some indication of tendency of decrease in the mean values of X_1, X_2, and X_4 and of

TABLE 1. Names and positions of rivers from which scales were collected, years sampled, sample sizes, mean discriminant values, standard errors of mean discriminant values, and sea ages of Atlantic salmon included in discriminant analysis.

River	(°N) Lat.	Year collected	No. fish	Mean discriminant value	SE	Sea age 1	2	3
Hunt	55°33′00″	1973	35	2.2836	0.2244	18	17	—
Sand Hill	53°33′00″	1974	50	1.3816	0.1877	31	19	—
Pinware	51°38′00″	19.4	38	1.8139	0.2153	10	28	—
Western Arm	51°11′25″	1974	35	0.5048	0.2244	35	—	—
Salmon	51°10′10″	1968	38	0.8249	0.2153	38	—	—
Indian	49°29′30″	1973	40	−0.6787	0.2099	40	—	—
Barney's	49°25′45″	1974	18	−0.0446	0.3129	18	—	—
Windmill	49°16′50″	1974	40	−0.9432	0.2099	40	—	—
White Bear	47°46′50″	1974	39	−2.0648	0.2126	39	—	—
Northeast Placentia	47°16′15″	1974	40	−2.8631	0.2099	40	—	—
Salmonier	47°10′40″	1974	37	−1.1728	0.2182	37	—	—
Northwest Trepassey	46°45′40″	1974	35	−3.2997	0.2244	35	—	—
Miramichi	46°45′00″	1973	50	−3.4444	0.1877	50	—	—
Miramichi	46°45′00″	1973	50	−2.2058	0.1877	—	49	1
Margaree	46°25′48″	1973	16	−1.7006	0.3319	16	—	—
Margaree	46°25′48″	1973	25	−2.4393	0.2655	—	21	4
North	46°16′12″	1973	12	−1.8062	0.3832	12	—	—
North	46°16′12″	1973	54	−2.5570	0.1806	—	53	1
St. Mary's	45°08′24″	1974	18	−5.1744	0.3129	18	—	—
St. Mary's	45°08′24″	1974	19	−5.1794	0.3045	—	12	7
Narraguagus	44°33′00″	1973	48	−5.2451	0.1916	–	46	2
LaHave	44°22′48″	1975	10	−6.0675	0.4198	10	—	—
LaHave	44°22′48″	1975	25	−6.5996	0.2655	—	23	2

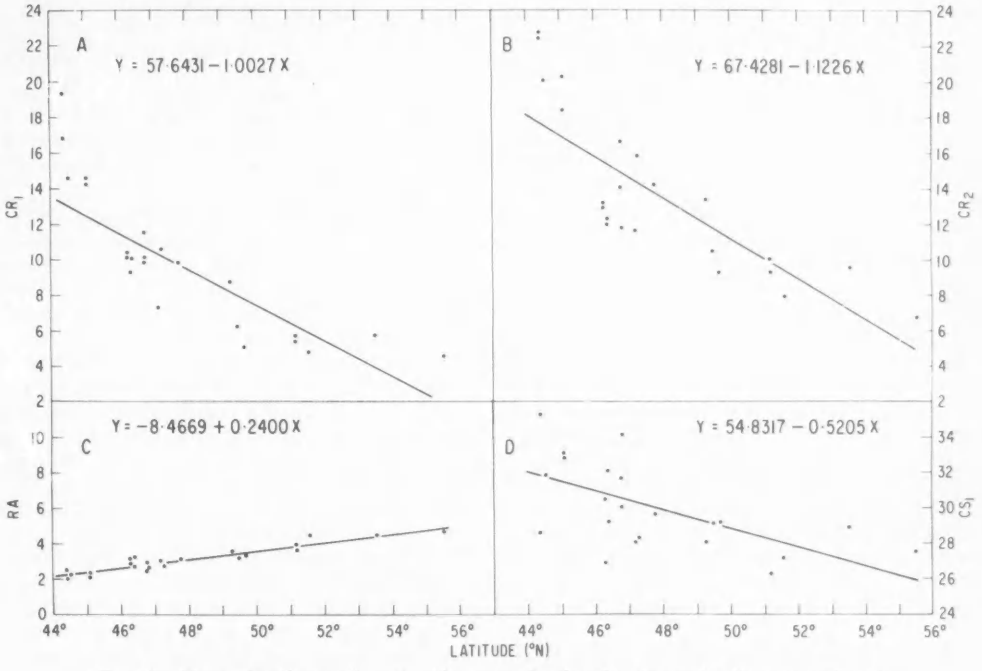

FIG. 2. Regression lines and scatter diagrams of points for four scale characters plotted against latitude (°N). A, $CR_1(X_1)$ = number of circuli in 1st river year; B, $CR_2(X_2)$ = number of circuli in 2nd river year; C, RA (X_3) = river age at smolt migration; D, $CS_1(X_4)$ = number of circuli in 1st sea year.

TABLE 2. Pooled within-sample correlation coefficients for four characters considered in pairs.

Character	X_1	X_2	X_3
X_2	0.37		
X_3	−0.33	−0.51	
X_4	0.10	0.19	−0.22

increase in the mean values of X_3 as we proceeded from south to the north. For the linear regression terms, mean squares were significant for all characters ($P < 0.01$, for each F on df = 1, 21). Table 2 gives pooled within sample correlation coefficients for all pairs of characters. These correlations were all significant ($P < 0.01$ for all pairs). There was also considerable overlapping of ranges of individual measurements for 23 samples.

In view of these preliminary observations, it seemed worthwhile to search for a combined criterion of four measurements that may provide a more effective separation between populations than a single measurement. Existence of the 23 reference samples was assumed (as the original classification was made independently of these four measurements) and so discriminant analysis was considered appropriate (Kendall and Stuart 1966). The objective of the present study was to investigate the cline as indicated by the linear trend of measurements on latitudes. This required the variation among samples due to only one comparison (viz the linear trend), and therefore, the discriminant function was determined by the technique (see eg. Williams 1959) that employs a "dummy" variate to obtain the maximization of the slope on the independent variable (in this case, the latitude). The discriminant function estimated was $Y = -0.2532X_1 - 0.1100X_2 + 1.3402X_3 - 0.0726X_4$. ANOVA based on the total corrected sum of squares for the independent variate as the dummy variate indicated that (a) the discriminant function and each individual discriminant coefficient contributed significantly ($P < 0.01$) to the clinal trend and that (b) a large fraction (73.7%) of the total variation was explained by this discriminant function. Table 1 gives the estimates of the mean clinal discriminant function and its SE (df = 746) for each area. Figure 3 shows a plot of these mean values against latitude. The trend indicated by these mean discriminant values was striking.

Analysis of covariance indicated that the slopes and positions of mean discriminant values on latitude did not differ significantly ($P > 0.05$) between the basic river data and tagged-recapture data. The linear regressions of mean discriminant values on latitude were as follows:

(1) Basic data: $Y = -39.0256 + 0.7716X$ (Fig. 3),

(2) Tagged–recaptured data: $Y = -31.4615 + 0.6129X$.

Discussion

The commercial fisheries for Atlantic salmon in Newfoundland–Labrador exploit mixed stocks of fish originating in river systems in Newfoundland, Labrador, the Maritimes, and Quebec and to a minor extent from Maine, USA. Results of tagging experiments have shown that the fishery in eastern and southern Newfoundland exploits to some degree salmon and grilse from the Miramichi River, N.B. (Turner 1972; Kerswill 1971; Blair 1956), Margaree River, N.S., Exploits and Harry's rivers, Nfld. (Blair 1956), Sand Hill River, Labrador (Peet and Pratt 1972), Quebec rivers (May 1973), and Maine rivers (A. L. Meister personal communication).

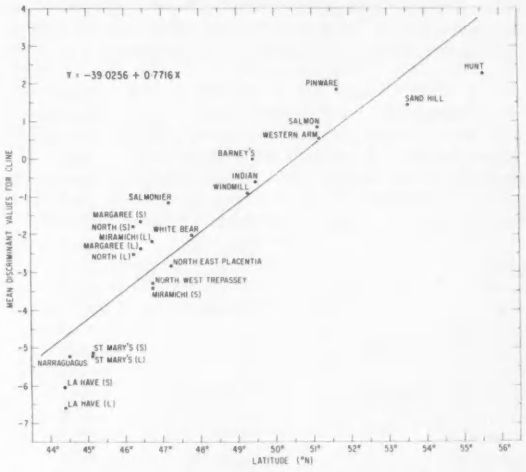

FIG. 3. Mean discriminant values for cline based on scale characters of Atlantic salmon plotted against the latitude of the river system from which scales were analyzed. S = small (1-sea-winter) salmon, L = large (2-, 3-sea-winter) salmon.

Several approaches, other than tagging, have been attempted in the past to determine areas of origin of fish exploited by the fishery. Based on the technique of serum electrophoresis, Payne (1974) stated that it is impossible to distinguish between Newfoundland and Nova Scotia populations of salmon on the basis of a discontinuity in the frequency of the *Tf4* allele. Pippy (1969) found that parasites as biological tags were of limited value in identifying individual salmon and because of low incidence of the parasites were of no value in identifying different Canadian stocks. The tagging–recapture method provides very valuable information but is extremely costly and usually results in only a small number of recaptures.

This paper demonstrates that there were highly significant differences between scale characteristics among samples of Atlantic salmon from northern Labrador to Maine. There was no difference between scale characters from grilse (small 1-sea-winter salmon) and large salmon from each of the three Labrador rivers sampled. Thus, in these northern rivers, there is no indication that there are differences in freshwater or

marine growth rates between salmon and grilse. In each of the five Maritime rivers analyzed, significant differences existed between scale characteristics of grilse and salmon. In all but the St. Mary's River, the 1st yr of marine growth was higher for salmon than grilse. For the St. Mary's River, the 1st yr of marine growth was not statistically different between grilse and salmon.

A latitudinal cline is evident in the four scale characters analyzed. The mean values for number of circuli in the first annual river zone, in the second annual river zone, and in the first annual marine zone increase from north to south, while the mean smolt age decreases from north to south. A clinal discriminant function was constructed using latitude as a "dummy" variable and this function explains 73.7% of the total variation.

Values obtained from the scales of salmon tagged from the mixed stock fisheries in the Newfoundland–Labrador area and eventually recaptured in the rivers from which they originated in eastern Canada have demonstrated that the clinal discriminant function is a valid method for separating the stocks.

Acknowledgments

We gratefully acknowledge the assistance of Mr T. R. Porter who provided scales from rivers in Newfoundland–Labrador, Mr G. Turner for scales from Miramichi, Mr R. Gray for scales from the LaHave and St. Mary's rivers, Dr T. L. Marshall for scales from the North and Margaree rivers, and of Mr A. L. Meister for scales from the Narraguagus River. Messrs F. A. Day and R. F. Burfitt read the ages and counted the circuli.

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Movements of Stream-Resident and Anadromous Arctic Char (*Salvelinus alpinus*) in a Perennial Spring on the Canning River, Alaska

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CRAIG, P. C. 1978. Movements of stream-resident and anadromous Arctic char (*Salvelinus alpinus*) in a perennial spring on the Canning River, Alaska. J. Fish. Res. Board Can. 35: 48–52.

Perennial springs in arctic drainages are used extensively by freshwater-resident and anadromous segments of Arctic char (*Salvelinus alpinus*) populations. Movements of these fish were monitored in a small spring on the Canning River from May 23 to October 7, 1973, dates approximating spring breakup and winter freeze-up in the drainage. Char passing through the weir ranged in size from 22-mm fry to 595-mm anadromous spawners, though most fish were juveniles measuring 60–200 mm. The spring channel was utilized as (1) a spawning area for anadromous spawners and residual males in autumn, (2) a rearing area for fry and some older juveniles in summer, and most importantly (3) an overwintering area for juvenile char, especially those in their first and second winters of life.

Key words: Arctic char, movements, spawning, overwintering, growth, perennial springs, Alaska, arctic

CRAIG, P. C. 1978. Movements of stream-resident and anadromous Arctic char (*Salvelinus alpinus*) in a perennial spring on the Canning River, Alaska. J. Fish. Res. Board Can. 35: 48–52.

Les segments anadromes et résidant en eau douce des populations d'ombles chevaliers (*Salvelinus alpinus*) utilisent largement les sources pérennes des bassins hydrographiques arctiques. Nous avons suivi les mouvements de ces poissons dans une petite source de la rivière Canning du 23 mai au 7 octobre 1973, dates qui correspondent à peu près à la débâcle printannière et au gel hivernal dans le bassin. Les ombles passant par la pêcherie fixe ont des tailles allant de 22 mm pour le fretin à 595 mm pour les reproducteurs anadromes, quoique la plupart des poissons soient des jeunes mesurant de 60–200 mm. Le chenal de la source est utilisé come (1) frayère pour les reproducteurs anadromes et les mâles résiduaires en automne, (2) aire d'alevinage pour le fretin et quelques jeunes plus âgés en été et, ce qui est plus important, (3) aire d'hivernage pour les jeunes ombles, surtout ceux qui en sont à leurs premier et deuxième hivers.

Received June 21, 1977
Accepted September 14, 1977

Reçu le 21 juin 1977
Accepté le 14 septembre 1977

IN Alaskan arctic drainages the integral relationship between perennial springs and the biology of Arctic char (*Salvelinus alpinus*) populations is now apparent. Spring-fed areas serve as spawning areas for this species and also as overwintering areas during winter when much of the remaining drainage may freeze solid. Beyond this general understanding, however, detailed information on utilization of springs by char is limited. This paper describes the summer movements of both stream-resident and anadromous char in a perennial spring on the Canning River.

The present investigation differs from other studies of char movements in several important respects. Two taxonomic forms of Arctic char occur in North America (McPhail 1961; McCart and Craig 1971), and most studies have concerned the movements (often lake-to-ocean migrations) of the Eastern Arctic form of *S. alpinus* in the Canadian arctic (e.g. Grainger 1953; Johnson and Campbell 1975; Moore 1975). In contrast, the Canning char represent the Western Arctic–Bering Sea form of *S. alpinus* (probably a distinct species), which is associated with rivers rather than lakes. Also, the Canning spring examined is utilized by stream-resident and anadromous members of the Canning population, unlike previously described dwarf populations of char that inhabit isolated arctic springs (e.g. McCart and Craig 1973).

Materials and Methods

In 1973 movements of Arctic char were monitored in a spring-fed channel on the Marsh Fork of the Canning River. Except for several periods of high water (indicated on Fig. 3), a fish weir was checked daily from May 23 to October 7, dates corresponding to the beginning of spring breakup

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Printed in Canada (J4892)
Imprimé au Canada (J4892)

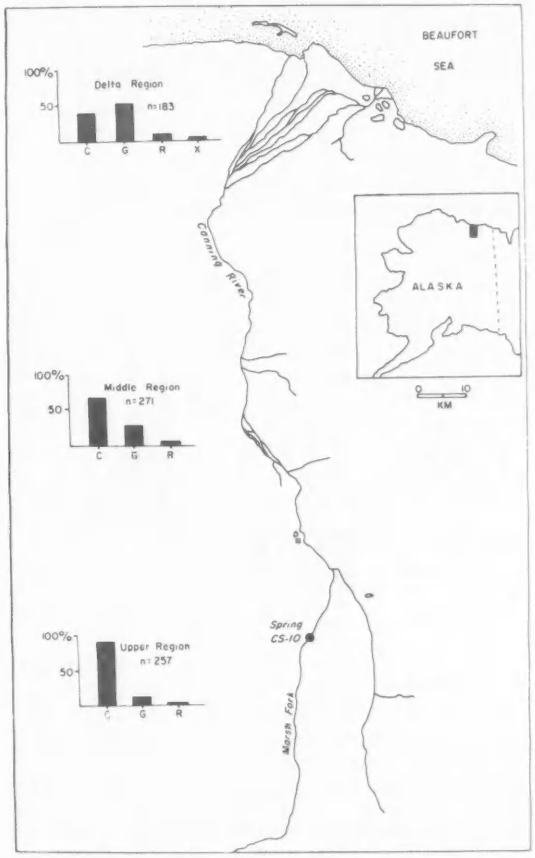

FIG. 1. Canning River showing location of Spring CS-10 and species composition of fishes caught by seine and gill net from delta to headwaters. Abbreviations used are C (Arctic char), G (Arctic grayling), R (round whitefish), and X (fourhorn sculpin, *Myoxocephalus quadricornis*; broad whitefish, *Coregonus nasus*; Arctic flounder, *Liopsetta glacialis*; Arctic cisco, *Coregonus autumnalis*; least cisco, *Coregonus sardinella*).

and the initial stages of freeze-up in the Marsh Fork adjacent to the perennial spring. The weir, located approximately 0.1 km above the spring mouth, completely blocked the spring with a small mesh (1.3 cm) wire screen placed diagonally across the channel to guide fish into holding traps (0.6-cm mesh) at either end. Captured fish were either measured and released or kept for laboratory analysis. Additional samples of anadromous char were taken by angling immediately downstream of the weir. Distribution information was also obtained by aerial surveys in 1972 and 1973.

Laboratory analysis of specimens included determination of fork length to the nearest 1.0 mm, sex, maturity, and age (otoliths). Gill rakers (total count, first arch, left side) and pyloric ceca were counted on a subsample of fish.

A general description of the Canning River, a moderate-size arctic drainage on Alaska's North Slope, is presented elsewhere (Ward and Craig 1974; Craig and McCart 1975; Craig 1977). Arctic char and Arctic grayling (*Thymallus arcticus*) are the dominant fishes throughout the drainage (Fig. 1).

TABLE 1. Age–length relationships for resident and anadromous Arctic char from Spring CS-10, 1972 and 1973. Data do not reflect size or age frequencies of the study population, since efforts were made to obtain fish from the less common size-classes.

	Resident char			Anadromous char		
		Fork length (mm)			Fork length (mm)	
Age	*n*	Mean(range)	SD	*n*	Mean(range)	SD
1	23	88(63–113)	13			
2	17	112(97–133)	11			
3	17	146(117–198)	21			
4	14	180(146–231)	27	1	299 —	—
5	12	201(157–252)	36	5	288(267–312)	16
6	12	245(177–296)	33	4	336(286–416)	49
7	14	242(211–280)	26	16	441(352–463)	25
8	5	281(225–318)	37	14	471(440–505)	21
9	4	274(240–331)	35	3	533(521–545)	10
10				2	496(478–514)	18
11	1	315 —	—	1	532 —	—
Totals	119			46		

The study area, designated Spring CS-10 (69°06'N, 145°59'W), is a small perennial spring originating on the steep bank of the Marsh Fork and flowing 1 km along the edge of the floodplain before discharging into the river. The actual length of the spring channel probably varies over the years as braided channels of the Marsh Fork meander and intercept the spring. In 1973 the lower half of the spring was subject to occasional flooding from the Marsh Fork, thus upsetting the spring's otherwise stable discharge (0.7–1.0 m^3/s), water temperature (4–6°C), channel width (5–15 m), and depth (10–40 cm). Seasonal fluctuations in discharge and water temperature in Spring CS-10 are presented by Craig and McCart (1975). In winter months when surface flow ceases elsewhere in the drainage, Spring CS-10 is a principal source of unfrozen water in this section of the Marsh Fork.

Results

STUDY POPULATION

Low gill-raker and pyloric ceca counts of anadromous and freshwater resident char in the Canning River identify these fish as the Western Arctic–Bering Sea form of *S. alpinus*. Anadromous char had an average of 22.0 gill rakers (range 20–24, SD = 1.4, $n = 52$) and 32.2 pyloric ceca (23–45, SD = 4.0, $n = 51$); resident char had 21.4 gill rakers (20–24, SD = 1.5, $n = 29$) and 30.1 pyloric ceca (18–42, SD = 5.0, $n = 31$).

Age–length relationships of freshwater-resident and anadromous segments of the Spring CS-10 char (Table 1) are similar to those observed in other areas of the Canning drainage (Craig 1977). However, the spring contained a higher proportion of older and larger fresh-

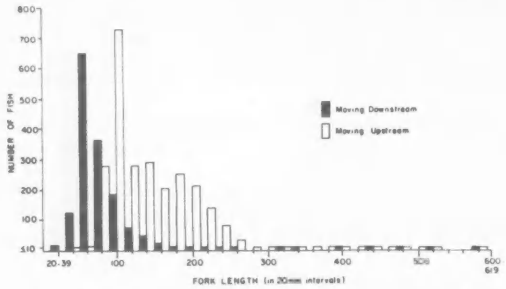

FIG. 2. Length frequencies of Arctic char moving in and out of Spring CS-10.

water residents than found elsewhere in the drainage. Anadromous char from the Canning tend to grow more slowly and average some 25 mm less at each age interval than other Western Arctic–Bering Sea populations in nearby Beaufort Sea drainages (McCart et al. 1972; Yoshihara 1972; Glova and McCart 1974; Bain 1974).

The category of freshwater residents includes presmolts and "residual" fish. The latter group mature in fresh water without ever undertaking a seaward migration. Residual fish are almost exclusively males and are identifiable by their small size (140–331 mm), coloration (various shades of spawning colors and the retention of parr marks throughout their lives), and sexual maturity. Some residual males were mature at age 3 and almost all by age 5 in the sample.

Most juvenile char in the Canning smolt between the ages 3–5 and enter coastal waters of the Beaufort Sea to feed. Anadromous char return to the rivers each fall to overwinter or spawn in spring-fed areas. Some Canning char spawn at age 6 but most mature later at ages 7 or 8 and spawn in alternate years thereafter. Peak spawning occurs from mid-September through October.

FISH MOVEMENTS

From approximately the time of spring breakup until freeze-up on the North Slope, virtually all fish moving in or out of Spring CS-10 were Arctic char (98.1% char; 4344 fish total). The remainder were grayling (1.8%) and round whitefish (*Prosopium cylindraceum*) (0.1%). The char ranged in size from 22-mm fry to 595-mm anadromous spawners, though most fish were juveniles measuring 60–200 mm (Fig. 2). Composition of the 4262 char enumerated at the weir was 1.0% anadromous spawners, 1.1% fry, 2.7% anadromous immatures, and 95.1% stream residents (including presmolts and residuals). Movements of these life-history stages are described below.

Anadromous char — Aerial surveys showed that Spring CS-10 was an important spawning area for Arctic char. On September 20, 1972 an estimated

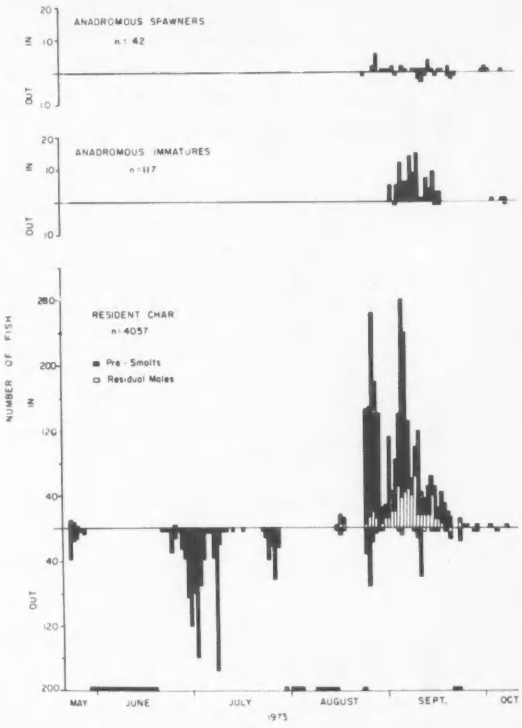

FIG. 3. Movements of Arctic char anadromous spawners, anadromous immatures, and freshwater residents (presmolts and residual males) into and out of Spring CS-10. Dark bars along abscissa indicate periods when the weir was not in operation. Note that ordinate scales differ.

1000–2000 anadromous spawners were sighted in the spring channel or river immediately downstream of the spring. By early winter (November 5, 1972) only half of these fish were sighted; the others had apparently dropped back downstream after spawning and were under ice cover. No sea-run char were sighted in open waters of the spring the following April, 1973.

In 1973 the first spawners appeared in the weir on August 24 and continued to enter the spring in small numbers throughout September (Fig. 3). Daily numbers of spawners remained low because the weir was located at the upper end of the spawning grounds (the main spawning grounds extended into the Canning mainstream downstream of the spring). Also, higher-than-usual autumn water levels in the Marsh Fork, and perhaps the weir itself, may have delayed the movements of some spawners into the spring.

The 42 anadromous spawners that did pass through the weir were 382–595 mm long. Female spawners were more abundant (60%) than males. No mature nonspawners (fish that had spawned previously but

would not spawn in the year of capture) were taken at this location.

Shortly after anadromous spawners began appearing in the weir, there was a small run of anadromous immature char. These were silver-colored fish with no parr marks. Most measured 210–290 mm long ($n = 117$) and were 4–6 yr old ($n = 9$). Spring CS-10 is the most inland site in the Canning drainage where anadromous immatures are known to migrate and presumably overwinter.

Resident char — Freshwater residents were by far the most numerous category (95.1%) of char using the spring. Residents were composed of an estimated 655 (16%) residual males and 3402 (84%) presmolts. These figures are approximate, however, because of the difficulty in distinguishing live presmolts from residuals. Only those residents showing definite spawning coloration were classified as residual males (this accounts for there being no residual males recorded among residents prior to the spawning season, as shown in Fig. 3).

Most residents were 60–200 mm long. The modal size was 60–79 mm for out-migrants in June–July and 100–119 mm for in-migrants in September (Fig. 2). The former mode corresponds to age 1 char early in the growing season, and the latter to age 1 char late in the growing season.

Some freshwater residents began leaving the spring in May (prior to the weir being washed out by spring flooding from the Marsh Fork) and a large-scale emigration took place in late June and early July (Fig. 3). Few fish moved in or out of the spring during the summer, and it was not until late August and September that there was a large influx of fish. The timing of these upstream movements appears synchronized with the immigration of sea-run fish. A drop in water temperature may have stimulated this behavior. Maximum water temperatures in the Canning River cooled rapidly from 11°C on August 22 to 6°C on August 28 and remained low thereafter.

Over 260 residents per day passed upstream through the weir during peaks on August 26 and September 5. A substantial run of residual males occurred at this time. These small fish, averaging 230 mm long (range 140–331 mm), accounted for 24% of all char entering the spring, and as previously mentioned, this percent is underestimated. By the end of September the number of residual fish and presmolts moving through the weir was negligible.

Char fry — Arctic char fry emerged from the gravel during the last week of May and first part of June 1973. They were abundant throughout the spring channel but few passed through the weir. Only 41 fry were caught leaving the spring (73% in July) and 6 entering it. Apparently most fry that emerged in Spring CS-10 also spent their first growing season in the spring channel. No immigration of fry from spawning areas below the spring was detected.

Discussion

Perennial springs, found in many Beaufort Sea drainages (Craig and McCart 1975), provide vital habitat for fish in the Western Arctic. Arctic char populations rely extensively on spring-fed habitats at particular stages of their life cycle. For example, Spring CS-10 is used as (1) a spawning ground for anadromous char and residual males, (2) a summer rearing area for fry and some older juveniles, and (3) an overwintering area for juvenile char, especially those in their first and second winters of life. This pattern is similar to that described for Arctic char in a Sagavanirktok tributary, the Lupine River, which has a single spring-fed area (Yoshihara 1972).

The spring appears to be an ideal rearing area for juvenile char. It provides a relatively stable discharge year round and supports a far greater food supply than occurs in the braided channels of the Marsh Fork or Canning rivers. Standing crops of benthic invertebrates ranged from means of 1279 to 6642 organisms/m^2 (predominantly chironomid larvae and ephemeropteran nymphs) in the spring channel compared to only 3 to 1077 organisms/m^2 in river channels (Ward and Craig 1974). Despite these features, many overwintering juveniles leave the spring channel in June and July, indicating that other factors such as crowding or lack of suitable cover must cause juvenile char to spend the summer elsewhere in the drainage. Where these fish actually go when they leave the spring is not clear, although surveys in the Canning and nearby drainages suggest a widespread summertime distribution. Juvenile char have been collected in springs, tributaries, rivers, and deltas of several North Slope drainages (McCart et al. 1972; Yoshihara 1972; Glova and McCart 1974; Bain 1974; Craig and Poulin 1975; Craig 1977). It is also not known whether out-migrant juveniles from Spring CS-10 are the same fish that return to the spring in the fall.

Movements of fry and older juveniles in the spring-fed area immediately downstream of Spring CS-10 are speculative. Presumably these fish disperse through the drainage when spring flooding in the Marsh Fork abruptly changes their small, springlike habitat in late fall and winter to a large, turbid river. Immigration from these downstream waters into Spring CS-10 apparently does not occur at breakup time.

Movements of anadromous char while in fresh water are understood in a general sense. Other workers have recorded an earlier arrival time of anadromous char onto spawning grounds than occurred at Spring CS-10. Migrants have appeared as early as mid- to late July in North Slope rivers, though most arrived in August (Yoshihara 1972; Bain 1974; Glova and McCart 1974). In view of these early arrival dates, there is some ques-

tion whether all maturing char undertake a seaward migration during summer prior to spawning. In any case, coastal studies show that most anadromous char return to fresh water by mid-August (Griffiths et al. 1975, 1977).

Tagging studies in the Sagavanirktok drainage suggest that anadromous spawners exhibit a high degree of homing to their spawning grounds (Furniss 1975). However, when anadromous immatures or mature char that are not in a spawning condition return to fresh water, they may overwinter elsewhere in the same drainage or occasionally in a different drainage altogether (Craig and McCart 1976). There is some direct evidence, then, that in large drainages all fish spawned in a particular spring-fed area do not necessarily return to that same location to overwinter each year. On the other hand, in the smaller North Slope drainages, which have few areas suitable for fish overwintering, it is conceivable that a single spring-fed site might harbor virtually all members of a particular char population, from eggs in the gravel to adult fish, during the 8–9-mo winter period.

Acknowledgments

I thank D. Ward, V. Poulin, R. Fuson, and G. Podsiki for their field assistance. Funding was provided by Alaskan Arctic Gas Study Co. (Anchorage, Alaska) and Canadian Arctic Gas Study Ltd. (Calgary, Alberta) through Northern Engineering Services Ltd. (Calgary, Alberta).

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Fish Population Structure in Arctic Lakes

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POWER, G. 1978. Fish population structure in arctic lakes. J. Fish. Res. Board Can. 35: 53–59.

Existing theories attempting to account for the predominance of older, larger individuals and the often observed bimodal character of the length-frequency distribution of unexploited northern fish populations were evaluated and rejected. The error in interpretation appears to result from problems in age determination of northern lake trout (*Salvelinus namaycush*) and lake whitefish (*Coregonus clupeaformis*). An alternative explanation of the observed structure of arctic lake fish populations is proposed. Growth of fish in these populations is relatively rapid until first maturity, when it becomes slow through a life span that may exceed 50 yr. Mortality declines rapidly through early life and stabilizes at a very low level through much of the life span. The combination of these growth and mortality patterns produces a population containing many small, few intermediate, and many large fish. Selection by gill nets provides a unimodal sample of large fish or a bimodal sample of large and smaller fish from such populations. A model is developed to demonstrate this and compared with results obtained from several northern Quebec lakes.

Key words: Salvelinus namaycush, Coregonus clupeaformis, age, population structure, arctic lakes

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Nous évaluons et rejetons les théories actuelles voulant expliquer la prédominance d'individus vieux et grands ainsi que le caractère bimodal, souvent observé, de la distribution des fréquences de longueur des populations de poissons septentrionales inexploitées. L'erreur d'interprétation semble résulter des difficultés à déterminer l'âge des touladis (*Salvelinus namaycush*) et des grands corégones (*Coregonus clupeaformis*) habitant ces régions. Nous proposons une autre théorie pour expliquer la structure des populations de poissons des lacs arctiques. Dans ces populations, les poissons croissent à un rythme relativement rapide jusqu'à la première maturité; la croissance ralentit par la suite et demeure lente pendant toute une vie qui peut dépasser 50 ans. La mortalité diminue rapidement au début et se stabilise à un très bas palier pendant la majeure partie de la vie. Ces types de croissance et de mortalité se combinent pour produire une population formée de nombreux petits poissons, de peu d'intermédiaires et de plusieurs grands poissons. La sélection exercée par les filets maillants fournit un échantillon unimodal des grands poissons et un échantillon bimodal des grands et des petits poissons des populations de ce genre. Nous élaborons un modèle démontrant ce phénomène et le comparons aux résultats obtenus dans plusieurs lacs septentrionaux du Québec.

Received June 14, 1977
Accepted September 15, 1977

Reçu le 14 juin 1977
Accepté le 15 septembre 1977

LAKE trout (*Salvelinus namaycush*) and lake whitefish (*Coregonus clupeaformis*) are generally the dominant species in arctic lakes and often account for close to 95% of the fish biomass (Johnson 1976). In many of these lakes the fish community is entirely, or virtually, unexploited. Catches are dominated by large fish of relatively uniform size producing a catch curve for length that is difficult to explain strictly on the basis of the selectivity of the fishing gear. Johnson (1976) provided a detailed overview of a wealth of information on size and age structure of samples gathered over many years from lakes in the Northwest Territories and proposed a hypothesis to explain the shape of the catch curve for length and apparent structure and stability of the fish populations. Briefly, his hypothesis is that the abundant large fish in northern populations dominate and suppress the younger, smaller members of the population. The subordinate fish grow slowly, tend not to show up in catch samples, and only filter through into the dominant class as vacancies arise due to death. Entry into the dominant class is followed by rapid growth and attainment of the size and comportment of dominant fish. This mechanism provides a highly sensitive intrapopulation control on recruitment and explains the long-term stability of northern fish stocks.

Printed in Canada (J4884)
Imprimé au Canada (J4884)

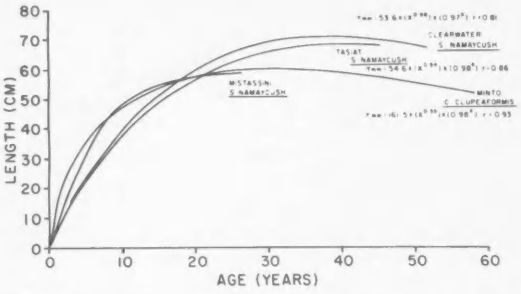


FIG. 1. Growth curves for three populations of *Salvelinus namaycush* from Lake Mistassini, Clearwater Lake, and Lake Tasiat, and for *Coregonus clupeaformis* from Lake Minto; the latter was derived from otoliths viewed in cross section.

The purpose of this paper is to point out some difficulties in accepting Johnson's hypothesis and provide an alternative explanation of the observed facts. The implications of the new interpretation in terms of population stability and the effects of exploitation are discussed.

Materials and Methods

The data published by Johnson (1976) and Dubois and Lagueux (1968) and collected by me from Clearwater lake (56°10′N, 74°30′W), Lake Minto (57°13′N, 74°53′W), and Lake Tasiat (59°11′N, 75°15′W) in northern Quebec are used to develop and support the hypothesis. The Quebec lakes were sampled by gill nets of stretched mesh 38, 51, 63, 76, 89, 101, 114, and 140 mm and of dimensions 91 × 1.8 m, fished singly from shore. The sampling technique and catches were similar to those described by Johnson (1976) for the Northwest Territories. Only lake trout and lake whitefish will be considered in the results.

Although scales are generally used for age determination in lake whitefish, burnt otoliths, cracked in median cross section through the nucleus, were used in this study. The technique was a modification of that outlined by Christensen (1964). The dry otoliths were placed on a hot plate at a temperature that caused them to brown in about 20 s. They were dropped hot into 95% alcohol, removed, broken in two, and viewed, end on, supported in plasticine while emersed in creosote oil. For a few, it was necessary to polish by hand the broken end surface using no. 600 carborundum wet paper. Lake trout ages were also determined by means of otoliths. For large fish it was sometimes necessary to polish the lateral surface to clarify the inner rings; the outer rings were occasionally clarified by burning as described above, but not by sectioning.

Age determination is difficult in long-lived, slow-growing arctic fish (Johnson 1976) and the problem is further complicated by the fact that material is not available to validate the results. Lake trout and lake whitefish are rarely captured during the first 2 or 3 yr in sufficient numbers that length-frequency distribution allows age-grouping and validation of annulii formed on scales or bony structures. In older fish interpretation of growth patterns on scales becomes very subjective and since no long-term (20+ yr) tagging experiments have been performed, impossible to validate. Other bony structures, such as otoliths, are usually easier to interpret and provide greater estimates of age in older fish but are even more difficult to validate. Scale and otolith ages agree for younger fish, and once annulus criteria are established for otoliths the greater ages derived from otoliths of larger fish are generally accepted as valid. This approach was used in this study. In lake trout otoliths, all the finer bands towards the tail of the otolith were accepted as annuli in agreement with the interpretation of Dubois and Lagueux (1968). Difficulties in interpreting the lateral surface of lake whitefish otoliths prompted the use of the technique outlined above and the discovery of the particular growth pattern of these otoliths described in the results. After burning, all dark bands except those obviously secondary rings common in the initial years were accepted as valid annuli.

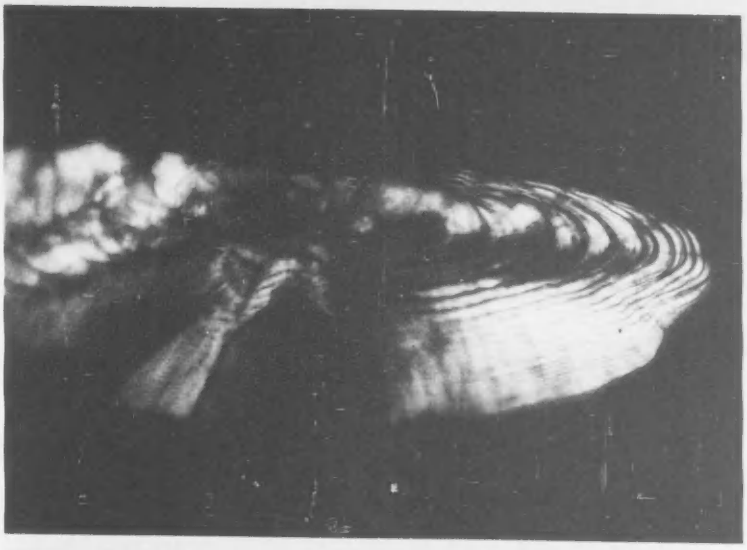
Results

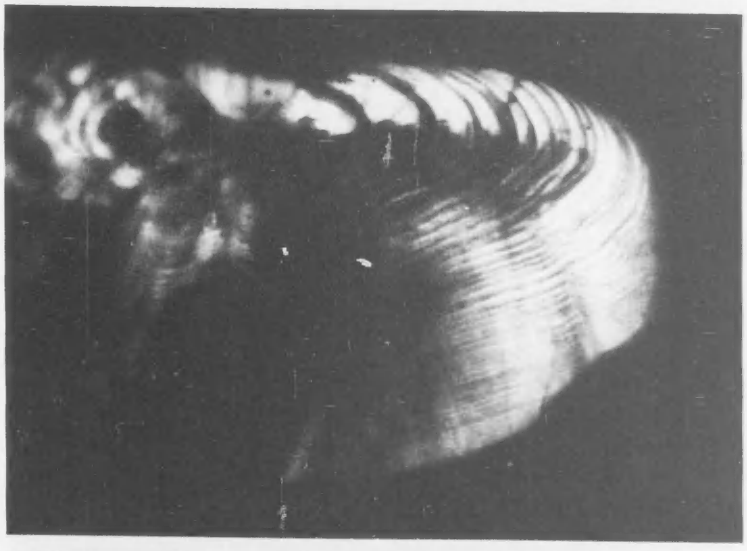
Age Determination

Dubois and Lagueux (1968) demonstrated the potential inaccuracy in aging trout from scales. They found discrepancies of a magnitude of two between scale age and otolith age among older trout. The growth curve derived from otolith ages flattens out beyond the age of first maturity, so that it assumes the form shown in Fig. 1, which includes Dubois and Lagueux's results for Lake Mistassini.

For lake whitefish the same order of discrepancy occurs between ages obtained from scales and those from otoliths; but the comparison is complicated as a result of the structure of the otolith. The pattern of deposition of annual layers around the nuclei of slow-growing lake whitefish otoliths is shown in median cross section in Fig. 2. For the first 3 or 4 yr the rings are complete, but much thinner on the surface opposite the central groove. For years 4 to 12 or 15, the rings are incomplete; they are interrupted by the groove and do not cover the opposite surface. This means the first 12–15 growth rings on lake whitefish otoliths, viewed laterally, are relatively easy to see. Up to this age, ages estimated from otoliths viewed laterally in median cross section or from scales agree closely. Beyond this age problems develop but otoliths viewed laterally and scales still tend to agree; unexploited northern populations have been assigned mean scale ages of 14–19 yr and maximum ages of 21–27 yr (Johnson 1976). In older fish crowded annuli at the periphery of the scale become impossible to decipher and the otolith ceases to grow around the lateral edges. New layers are added to the otolith only as caps on either side of the central groove

FIG. 2. Broken median cross sections of burnt otoliths of lake whitefish aged 20 *upper panel*, 42 *middle panel*, and 57 *lower panel*. All otoliths are similarly oriented; note the increase in thickness of the otoliths with age resulting from incomplete growth bands.





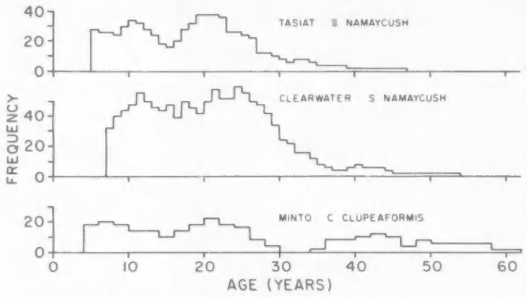


FIG. 3. The age-frequency histograms of samples of *S. namaycush* from Lake Tasiat and Clearwater Lake, and of *C. clupeaformis* from Lake Minto. The curves have been smoothed by running averages of 5 and the frequencies multiplied by 10 to eliminate random fluctuations caused by small numbers.

and these are not visible when the otolith is viewed laterally (Fig. 2). Even on fish as young as 20 yr different ages will be assigned when otoliths are viewed laterally (Fig. 2, *upper*) or in cross section. Otoliths of fish older than about 15 yr become thicker and more robust, rather than increasing in length or width, as can be followed in the sequence in Fig. 2. Otoliths of old fish can easily be picked out from those of young fish by their robust form.

When scales or otoliths are examined laterally, in the traditional manner, they will generate quite different ages for old fish than when the otolith is examined in cross section. Likewise growth curves, catch curves of age, and estimates of mortality will be quite different.

CATCH CURVES

Catch curves or age-frequency distributions, biased by errors in age determination, will tend to concentrate fish in the age-groups where the method of age determination breaks down. When plotted these curves tend to be leptokurtic or approach normal (Johnson 1976). When fish are aged by the criteria used in this study, the age-frequency distributions are platykurtic with a positive skew (Fig. 3). Such curves indicate extremely low mortality rates over much of the life span. In some northern Quebec lakes there is evidence of an increase in the mortality rate of the oldest lake trout (J. Benoit, Centre d'Etudes Nordique, Univ. Laval, personal communication); egg production rates indicate that mortality rates must be much greater among the youngest age-classes. The lifetime survivorship curve then follows a form not unlike that of a mammalian species, except that because of fecundity differences the first segment is steeper.

MODEL OF POPULATION STRUCTURE

If a growth curve typical of unexploited northern stocks of lake trout or lake whitefish is combined with a

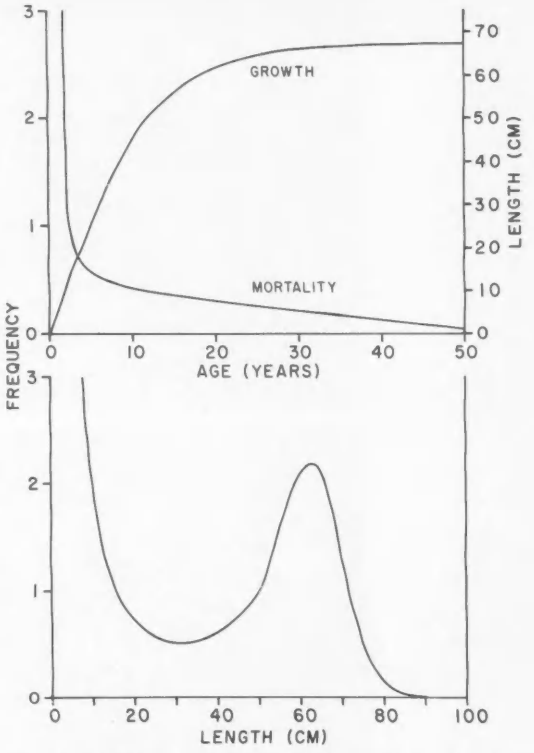


FIG. 4 Model of arctic lake fish population structure. *Upper panel* shows the form of the growth curve and the pattern of mortality as indicated by the age-frequency distribution. *Lower panel* is the length-frequency distribution of the population when the coefficient of variation of length with age is taken as 10.

survivorship curve in which an initial high mortality rate declines rapidly to a low rate for most of the life span, the length frequency distribution of the resulting population has high numbers of very small fish, few fish of intermediate size, and large numbers of fish clustered around the average maximum size of the species (Fig. 4). In constructing the figure to illustrate the general situation, lengths and frequencies for each age-group were read from the graphs (*upper panel*) and length-frequency distributions were calculated on the assumption that these were normal distributions with SD = 0.1 of the mean. This value appears to be a reasonable average for age–length data in fish and was chosen after examining a selection of published figures in the literature. Age-specific length frequencies, by 5-cm intervals, were then summed to give the population length-frequency distribution (*lower panel*). The conformity of the model to actual populations is reasonable and within the usual limits of measurement errors for length and age (Fig. 5) and mortality (Fig. 6). In estimating mortality for lakes Tasiat, Clearwater, and Minto a

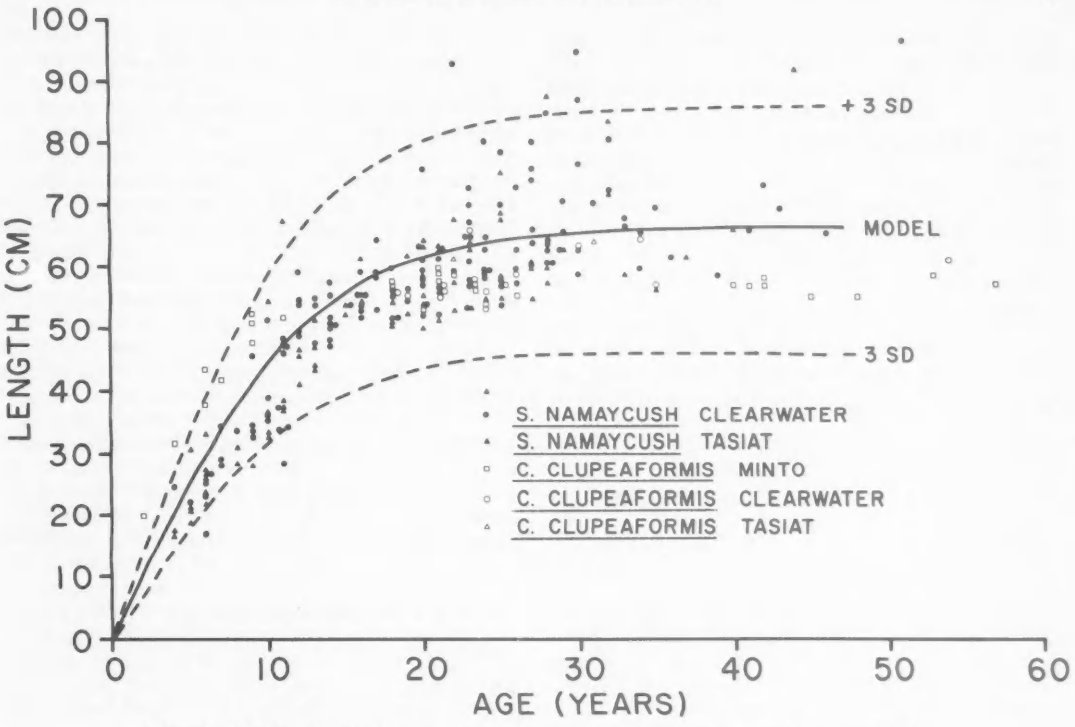


FIG. 5. Conformity of the model growth curve with observed age–length data for *S. namaycush* from Lake Tasiat and Clearwater Lake and *C. clupeaformis* from lakes Minto, Clearwater, and Tasiat.

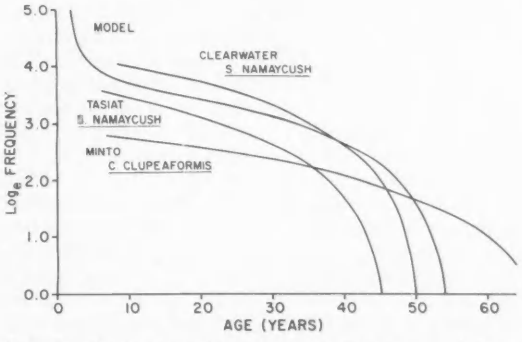


FIG. 6. Conformity of the model mortality rates with mortality rates observed for *S. namaycush* from Lake Tasiat and Clearwater Lake and *C. clupeaformis* from Lake Minto.

straight line was fitted to an arithmetic scale of catch frequency against age. Because of small numbers in any age-group the frequencies were first smoothed by using running averages of frequencies over five age-groups. For observations from age 8 upwards this gave a fit ($r = 0.7$) that was similar to that obtained using a logarithmic scale of frequency and has the advantage of implying a mortality rate that increases gradually with age. This seems biologically more reasonable than assuming a constant rate of mortality. For the purpose of the model either pattern gives essentially the same result because between ages 10 and 40 the curves are almost identical.

Discussion

Catch curves that approach normal or are leptokurtic such as those described by Johnson (1976) indicate high mortality rates for the descending right-hand limb of the curve. If these mortality rates are extrapolated backwards to the younger age-groups, they suggest a relatively high proportion of the fish present in the population have escaped sampling by the gear. Since in arctic populations these fish may be quite old and, if growth curves derived from the sampled part of the population are accepted, quite large, it is difficult to explain this bias. Either a complex mortality curve or a complex growth pattern has to be hypothesized. These difficulties do not arise when catch curves are platykurtic and skewed to the right.

Johnson (1976) hypothesized a complex growth process to explain his observations but presented little evidence to support it. If growth were highly variable

throughout the life of arctic fishes, one would expect records of changes in growth rate to be visible on scales and/or otoliths. These changes could then be measured using one of the established methods of back-calculation. In material examined from northern Quebec there is no indication of the kind of changes in growth rate required to support Johnson's hypothesis. If it is accepted that gross errors in aging have occurred, it becomes unnecessary to hypothesize complex growth patterns.

A less extreme example of the model developed here to explain the structure of arctic lake fish populations is provided in Ricker (1975, fig. 2.11) following Baranov (1918). Baranov developed his model to demonstrate that length-frequency distributions were not always reliable indicators of mortality. It involved a steady low mortality rate combined with a growth rate that fell by half from age 7 onward and produced a length-frequency distribution that did not decline steadily with length. This explanation of the structure of arctic fish populations has escaped notice for so long because there have been too many errors in age determination, and when exploitation occurred the growth curve was truncated and the mortality pattern changed so that the situation was less evident.

Given the interaction between growth and mortality in influencing the size distribution and recognizing that the smallest fish are underrepresented in catch samples, it is easy to see the origin of either a bimodal or a unimodal size distribution that approaches normal. The prevalence of leptokurtic length-frequency distributions in Johnson's (1976) data is difficult to explain unless fish of a particular size are more vulnerable to capture by the methods employed. If, superimposed on a population structure described by Fig. 4, there is a behavioral component involving slightly greater activity of fish of modal length, there would be a bias in favor of fish of modal length in gillnet catches that depend on fish movement to function. This would be sufficient to produce a leptokurtic length-frequency distribution.

Population Stability

The model of population structure developed here presupposes a low mortality rate for a large segment of the life cycle. The ecological consequences of this are to provide stability in the face of climatic fluctuations, which at intervals may exceed the limits for successful reproduction of the species.

Lake trout population dynamics are easiest to understand. The reproductive potential of the population provides an excess of young, even though females do not generally spawn every year. These young are important in the overall dynamics of the community because they can forage for small food organisms not generally available to, or which cannot be efficiently utilized by, large fish. Excess young are removed by intraspecific density-dependent predation and possibly by competition for resources in years of successful spawning. Because of the longevity of the species this mechanism provides a highly damped feedback control on recruitment. The high proportion of old fish in the population, all capable of reproduction, provides a reproductive reserve that can tolerate even a succession of years when the climate is unfavorable for reproduction. The advantage of multiple spawning in providing stability to populations was recognized and discussed by Ricker (1954). More recently Murphy (1967, 1968) has amply demonstrated the value of iteroparity in dampening the effects of environmental fluctuations, using theoretical argument and his analysis of the Pacific sardine fishery. Two consecutive environmentally induced spawning failures occurring at a time when exploitation had reduced the possibility of multiple spawning from 7 or 8 to 2 led to the collapse of the fishery. In northern lake trout populations poor years for reproduction will be reflected in the age-frequency distributions of samples of the populations, but in small samples they are difficult to distinguish from random variations due to sampling and in earlier work they have probably been masked because of errors in age determination using scales. Once recognized, and this depends on precision in age determination, variations in year-class strength will provide a powerful clue to understanding the biology of northern lake trout populations. Conditions in summer appear at first sight to be the most critical, but whether a good summer leads to a higher proportion of the adult stock becoming sexually mature or a poor summer leads to high mortality of young is not immediately apparent. The efficiency of this feedback control and the fact that intraspecific predation allows lake trout to exploit energy available at many points in the ecosystem probably go a long way toward explaining the success of the species in the north.

Lake whitefish populations cannot be stabilized by the same mechanisms proposed for lake trout because there is no evidence of intraspecific predation. They produce many small eggs and the early stages of life are subject to heavy mortalities. These can be density dependent, hence regulatory, for example, as in predation by lake trout or northern pike (*Esox lucius*), which generally occur with whitefish; or density independent, such as winds and currents preventing fry arriving at the best nursery areas (Hoagman 1973). These factors reduce year-class strength early in life but more sensitive control mechanisms are likely at the level of fecundity and frequency of reproduction of the adults. Adult whitefish certainly do not spawn every year in arctic lakes but suitable data on the frequency of spawning are not available. Intraspecific interactions perhaps at the level of competition for resources may control spawning frequency, and this can be much more flexible and responsive to immediate conditions than changes in individual fecundity per maturation cycle. The latter provides a long-term adjustment to average local conditions.

Exploitation

Apart from understanding basic ecological mechanisms, interest in northern stocks of freshwater fish has centered around their use in either domestic or com-

mercial fisheries. As Johnson (1976) points out, these stocks are particularly attractive because they constitute a large biomass of big fish. Exploitation at a low rate can take place without endangering the stock; the same control mechanisms that bring about stability of the population are sufficiently robust to allow this. The danger arises when exploitation increases to the point that the reserve of old fish in the stock begins to be depleted. In southern areas, where the chances of successful reproduction every year are high, one, reproducing year-class (or at the most two) is sufficient to maintain a population. In the north, the number of year-classes required increases with the probability of successive bad years for reproduction. At the extreme northern edge of the range, when reproduction becomes critical, many year-classes are required to ensure the long-term survival of a species. The important question is that of time scale. A species may be able to tolerate moderate exploitation for many years only to collapse suddenly when additional stress in the form of a series of unfavorable years is applied (Murphy 1967). Therefore great caution and restraint should be exercised in the management of northern fisheries.

Acknowledgments

I thank L. Johnson for several stimulating discussions on arctic fish populations, and R. J. Beamish for reinforcing my suspicions about possible problems in age determination of arctic fish. Richard Boisvert assisted in data analysis. R. L. Haedrich and W. C. Leggett read the manuscript and offered some useful suggestions. The research was funded by grants from the National Research Council of Canada; the Quebec Ministry of Education and the Centre d'Etudes Nordiques, Université Laval.

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Analysis of Growth of Atlantic Salmon (*Salmo salar*) in a Small Newfoundland River

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CHADWICK, E. M. P., T. R. PORTER, AND P. DOWNTON. 1978. Analysis of growth of Atlantic salmon (*Salmo salar*) in a small Newfoundland river. J. Fish. Res. Board Can. 35: 60–68.

Growth and sea survival rates decreased with increasing smolt age, with survival being 12, 6, and 3% for 3+, 4+, and 5+ smolt, respectively. All spawning fish were grilse, which suggests that older smolt became large salmon and were thus more vulnerable to the commercial fishery. A density-dependent relationship was observed for 3+ smolt in their 1st yr of growth, but not for older smolt; younger smolt probably spend their juvenile life in a more productive but space-limiting part of the river. Variation between river-system environments may be responsible for the opposing results of studies on Atlantic salmon (*Salmo salar*) life history.

Key words: Salmo salar, growth, sea survival, density dependence, first-year growth, age at smoltification

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Le rythme de croissance et la survie en mer diminuent en fonction de l'âge des smolts, la survie étant de 12, 6, et 3% pour des smolts d'âge 3+, 4+, et 5+ respectivement. Comme tous les reproducteurs sont des grilses, nous croyons que les smolts plus âgés deviennent de grands saumons et sont ainsi plus vulnérables à la pêche commerciale. Nous avons observé une relation dépendante de la densité chez des smolts de 3+ dans leur 1re a de croissance, mais non chez les smolts plus âgés; nous mettons de l'avant l'hypothèse que les jeunes smolts passent leur stade juvénile dans un secteur de la rivière productif mais d'espace limité. Les différences dans l'environnement d'un réseau fluviatile à l'autre peuvent être responsables des résultats contradictoires d'études sur le cycle biologique du saumon atlantique (*Salmo salar*).

Received June 2, 1977
Accepted September 9, 1977

Reçu le 2 juin 1977
Accepté le 9 septembre 1977

ALTHOUGH information on juvenile sizes at various ages (Power 1973), size at smoltification (Sedgwick 1953; Elson 1957), marine growth and size at return (Allen et al. 1972; Saunders and Allen 1967), seasonal pattern of adult return (Saunders 1967), and growth of landlocked Atlantic salmon (*Salmo salar*) (Leggett and Power 1969) is available, little is known of growth differences between ages at smoltification in a single year-class. Some authors have reported negative correlations between duration of river and sea life (Hutton 1937; Ritter 1974), while others have found no such correlations (Dahl 1937, Shearer 1966). Furthermore, growth rates of grilse with different river ages have not been quantified, and little is known about the possibility of density-dependent growth during the river life of migratory populations.

This paper examines sea and river growth for a salmon population in a small Newfoundland river. Variations in growth between fish of different ages at smoltification and of different year-classes are tested and related to sea survival and population size.

Printed in Canada (J4865)
Imprimé au Canada (J4865)

Study Area

Western Arm Brook flows into St. Barbe Bay on the northwestern coast of insular Newfoundland (51°11′25″N, 56°45′58″W), has a drainage basin of only 150 km^2, an axial length of 27 km, and its headwaters contain numerous shallow ponds and lakes. The river lies on Ordovician bedrock, which contributes to its relatively high specific conductance of 109 $\mu mhos \cdot cm^{-1}$ (when compared to the very soft water, about 38 $\mu mhos \cdot cm^{-1}$, found throughout most of Newfoundland (Jamieson 1974)). Daily temperatures, water levels, and smolt and grilse counts have been recorded in several data reports (Traverse 1971, 1973; Porter and Davis 1974; Pepper et al. 1975).

Materials and Methods

In May of each year, 1971–76, a 100-m portable counting fence (Anderson and McDonald 1977) was installed

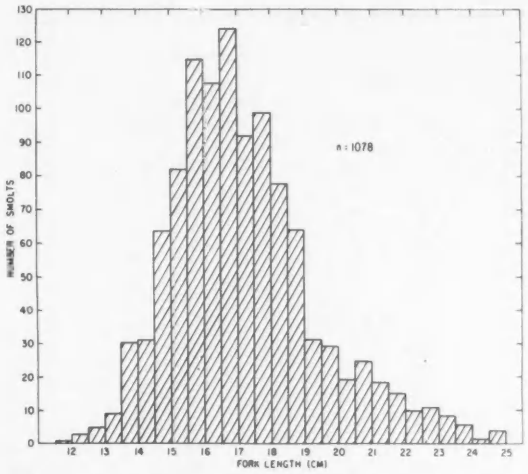

FIG. 1. Length-frequency distribution for all smolts sampled in Western Arm Brook (1971–76).

100 m upstream from the river's mouth to monitor the smolt and adult salmon migrations. Traps were checked several times daily, and nightly when necessary: fish were counted; some were transferred (see Traverse 1973; Porter and Davis 1974; Pepper et al. 1975) and the remainder allowed to continue their migration. During the smolt run, 5 smolts were sampled at the end of every 200 that had passed through the trap. These fish were anesthetized, measured for fork length and weight, and scale samples taken from the region between the lateral line and dorsal fin. Only 7% of the sampled smolt were killed for sex determination. Length, weight, and scale samples were taken and sex determined for practically all salmon angled. Sampling was continued each year until the end of the adult migration. Rates of survival from smolt to escaping grilse were calculated for each year-class and combinations of year-classes for given years.

Fifty-five percent of grilse scales and 85% of smolt scales were aged, and 56 and 43% of these, respectively, were randomly selected for back-calculation so that all years of capture would be represented with a sample size of at least 60. Scale annuli were discerned by standard criteria (Havey 1959; Berg and Grimaldi 1967) and their distances were measured from the focus along the longest oral radius (Tesch 1968). Linear relationships between total scale radius and fork length were determined for smolt and adults for each year of sampling. The yearly regressions were found to be homogenous (Snedecor and Cochran 1969) and those for 1972, which offered the highest r^2, were used for back-calculating fish lengths. The equation for the relationship between fork length of grilse and scale radius was used only for calculating the sea-year growth; the relationship between length of smolt and scale radius was used for determining freshwater growth. Back-calculated lengths were calculated for year-classes of grilse and smolts.

The relationship between weight and fork length was established for all samples of smolts and grilse combined. Values obtained from embryonic fish (D. Meerburg, Department Fisheries and the Environment, Fisheries and Marine Service, St. John's, personal communication) were also included in establishing the equation. Lengths back-calculated for younger ages were used to obtain estimated weights at younger ages. Instantaneous growth rates (Ricker 1975) were then calculated for the yearly weight increments computed above.

TABLE 1. Annual counting-fence enumeration (1971–76) of smolts, grilse, and kelts. Spawning grilse were fish that escaped angling harvest and transfer.

Year of capture	Smolts	Grilse	Spawning grilse	Kelts
1971	5,734	937	732	185
1972	11,906	417	253	210
1973	8,484	787	340	95
1974	12,055	504	297	302
1975	9,636	739	508	199
1976	6,259	552	416	208
Mean	9,012	656	424	200

Analyses of variance were utilized to test for differences between the following: (1) 1st yr of growth (l_1) for smolt and grilse at three smolt ages; (2) sea growth for grilse with different smolt ages; (3) fork lengths for escaping grilse of different smolt ages; (4) lengths at smoltification for 3-, 4-, and 5-yr smolts; (5) 1st yr of growth (l_1) for fish from different year-classes; (6) relationship between 1st-yr growth and year-class size for each age-group of smolt.

Results

The length frequency for all smolts counted (1971–76) approached a normal distribution with a mode of 17.5 cm (Fig. 1). The mean annual number of smolts was 9012 (SD ± 2706), for the adult escapement 656 (SD ± 195) and the kelt run 200 (SD ± 73); the standard deviation was roughly 30% for all totals (Table 1). Most of the smolt migration occurred during the first 3 wk of June, while the peak of the adult migration was usually in early August. The sex determinations indicated that 79% of 80 smolts sampled and 76% of 224 adults sampled were female.

A highly significant relationship ($r^2 = 0.96$) existed between number of returning grilse and number of outmigrant smolts from the preceding year. The equation was $Y = 226 \exp(9.9 \times 10^{-5}X)$, where Y = grilse in hundreds and X = smolts in thousands. The lowest smolt to grilse survival rates were found at intermediate smolt densities, viz. 5.9 and 5.7% for the 1973 and 1975 smolt year-classes, respectively, while the highest survival rate of 7.3% was found for the smallest smolt class in 1971.

Examination of survival rate by age-group (Table 2) indicated that fish that smoltified at age 3 usually had the greatest chance of surviving their sea year and returning to their natal river as grilse, while those at age 4 had an intermediate survival rate of 6.4%; except in 1973, fish that smoltified at age 5 had the lowest chance. The smolt to grilse survival rate by year-class was established by combining all fish with the same

TABLE 2. River age compositions of smolts leaving river and of grilse returning to river the following year, and survival rates for each group.

Year of smoltification	Smolt age-group	Smolts		Grilse		Survival rate (%) (smolt to grilse)
		No.	% total	No.	% total	
1971	3+	975	17	126	30	12.9
	4+	3,153	55	226	54	7.2
	5+	1,318	23	58	14	4.4
	6+	287	5	8	2	2.8
1972	3+	1,071	9	118	15	11.0
	4+	8,929	75	621	79	7.0
	5+	1,786	15	47	6	2.6
	6+	119	1	—	—	0
1973	3+	1,273	15	116	23	9.1
	4+	6,701	79	318	63	4.7
	5+	509	6	70	14	13.8
1974	3+	1,326	11	200	27	15.1
	4+	7,594	63	525	71	6.9
	5+	2,893	24	—	—	0
	6+	241	2	15	2	6.2
1975	3+	2,601	27	552	(24)[b]	7.8
	4+	7,034	73		(67)	
Total[a]	3+	4,645	13	560	23	12.1
	4+	26,377	69	1,690	69	6.4
	5+	6,505	17	175	7	2.7
	6+	647	2	23	1	3.6
Overall survival		47,809		3,000		6.3

[a]1975 data not included in total.
[b]Estimated.

TABLE 3. Smolt to grilse survival rate, instantaneous mortality and instantaneous growth for 1967–71 year-classes; back-calculated mean length at end of 1st-yr growth (l_1) also included.

Year-class	No. smolt	No. grilse	Survival (%)	l_1[a] (cm)	Smolt to grilse Mortality (z)	Growth (G)
1967	4,938[b]	273	5.5[c]	4.61	2.90	—
1968	10,634	832	7.8	4.66	2.55	3.41
1969	10,665	436	4.1	4.78	3.20	3.66
1970	8,867	641	7.2	5.17	2.53	3.19
1971	8,360	586[d]	6.8	5.11	2.69	3.01

[a]No significant difference between years: $F = 1.65$, $P = 0.2$.
[b]3+ smolt unknown and not included.
[c]3+ smolt and 4+ grilse omitted.
[d]Estimated by assuming same proportion at each age as in previous years.

year of hatching regardless of year captured (Table 3). The lowest (4.1%) and highest (7.8%) survival rates were found in the largest year-classes. Survival of kelts from spawning grilse ranged from 29% in 1971 to 88% in 1973, with an overall mean of 63% (Table 1).

There was a good correlation ($r^2 = 0.91$) between scale radius and fork length for 1972 smolts (Fig. 2), while a low correlation ($r^2 = 0.54$) was observed for 1972 grilse. The back-calculated values were tabulated separately for smolts (Table 4) and grilse (Table 5).

The equation calculated and used for estimating fish weight from length was $Y = 1.5 \times 10^{-5} X^{2.95}$, where Y is weight (grams) and X fork length (millimetres).

There was an inverse relationship between length at the end of the 1st-yr growth and smolt age. Fish that smoltified at age 3 grew significantly ($P = 0.001$) larger in their 1st yr than those that smoltified at older ages (Table 4). Although the same trend could be seen among grilse (Table 5), the length at age difference was not significant ($P = 0.16$). An overall comparison between grilse and smolts revealed a significant difference for l_1 ($P = 0.01$), but not for l_2 ($P = 0.12$) nor l_3 ($P = 0.71$). Lee's phenomenon, where the back-calculated lengths at a certain age are smaller for older fish (Ricker 1975), was evident for smolts and grilse in the older age-groups.

Length at smoltification increased slightly with smolt age (Fig. 3) but not significantly ($P = 0.07$). This suggested that the faster growth seen in the larger l_1 for younger smolt continued throughout freshwater life (Fig. 4). Similarly there was no significant difference ($P = 0.20$) between the sea growth for escaping grilse of different smolt ages.

No relationship between 1st-yr growth (l_1) and smolt year-class size was immediately perceivable when all smolt ages were combined (Table 3). However, when

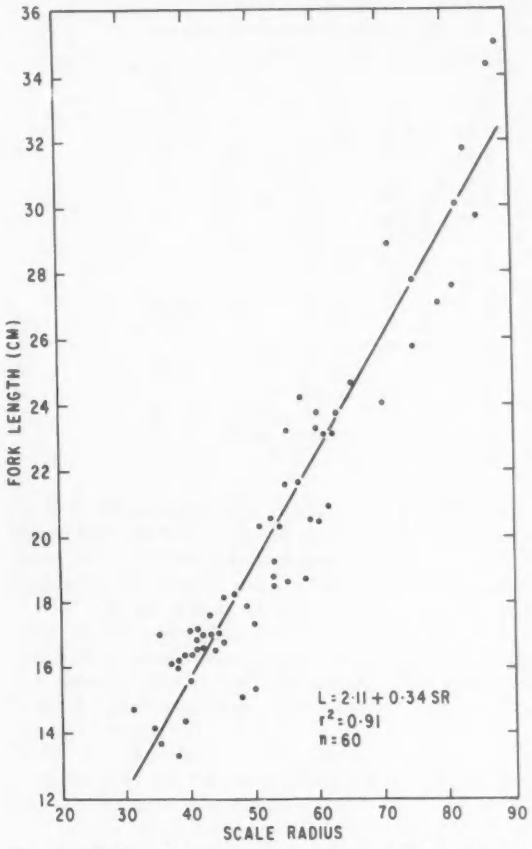

FIG. 2. Relationship between fork length of smolt and total scale radius (millimetres × 40).

the three smolt ages were treated separately, density-dependent growth was suggested (Fig. 5). Apparently, while the l_1 for 4+ and 5+ smolts increased with increasing year-class sizes, the 1st-yr growth of 3+ smolts decreased.

Discussion and Conclusions

The salmon population in Western Arm Brook could be characterized by several features. Most strikingly, the entire spawning population was composed of grilse, and like the smolts, they were predominantly females. Although the dominant age at smoltification was 4, salmon that smoltified at a younger age not only had grown faster but were also more likely to survive their sea year and return to the river as spawners; 5-yr smolt had the slowest growth and lowest sea survival rate. There was also an indication of different types of density-dependent 1st-yr growth for the three smolt ages.

The adult sex ratio found in our study appears to be an exception, as many studies indicate that grilse are predominantly males throughout most of the geographic range of this species (Berg 1959; Pyefinch 1969). Pratt et al. (1974) reported that 88% of the escaping grilse to Sand Hill River, Labrador, were male. Ruggles and Turner (1973) found that males outnumber females by 4:1 in Miramichi River, N.B. However, work in insular Newfoundland agrees with the present findings of a 75% female grilse component (Davis and Farwell 1975).

There is little information available on the sex ratio of smolts. In Newfoundland a preponderance of females was found on Exploits River (77%; Davis and Farwell 1975) and Sand Hill River (60%; Pratt et al. 1974). Several European studies also found that smolts were mostly females (Pyefinch and Mills 1963; Oster-

TABLE 4. Back-calculated fork lengths (cm) of smolts by year-class and smolt age.

Year-class	Smolt age	Sample size	Mean length (cm)	Back-calculated mean length				
				l_1	l_2	l_3	l_4	l_5
1973	3	8	15.8	5.78	10.37	14.83		
1972	3	22	16.5	5.37	9.96	15.47		
	4	39	17.3	5.24	9.11	13.87	18.06	
1971	3	18	16.6	4.81	9.29	13.99		
	4	59	17.4	5.17	8.77	12.92	17.14	
	5	10	20.2	4.86	7.89	12.41	16.39	20.40
1970	3	12	16.8	5.54	9.39	13.83		
	4	26	17.9	5.05	9.08	13.58	17.12	
1969	3	12	16.6	5.82	10.17	15.78		
	4	50	16.9	4.87	7.86	11.49	15.32	
	5	10	18.3	4.56	6.94	10.07	13.88	16.68
1968	3	7	16.8	5.24	11.04	18.36		
	4	50	22.5	5.32	8.85	14.57	20.03	
	5	5	15.9	4.35	6.96	9.57	12.83	15.88
1967	4	29	16.9	4.76	7.40	11.17	15.87	
	5	25	24.6	4.70	7.74	12.34	17.79	22.71
1966	5	15	20.1	4.58	6.94	10.02	14.65	18.65

TABLE 5. Back-calculated fork lengths (cm) of grilse by year-class and smolt age.

Year-class	Smolt age	Sample size	Mean length (cm)	Back-calculated mean length					
				l_1	l_2	l_3	l_4	l_5	l_6
1971	3	5	54.8	5.44	11.02	17.48	48.65		
	4	11	53.2	5.27	9.05	13.98	18.40	51.10	
1970	3	13	52.0	4.85	9.24	15.64	47.44		
	4	13	53.1	5.25	8.62	13.07	17.44	50.61	
1969	3	18	51.2	4.41	7.85	12.04	45.16		
	4	30	52.8	4.26	7.21	10.95	15.02	47.90	
1968	3	24	53.4	4.83	8.68	14.60	47.22		
	4	49	53.1	4.28	6.80	10.52	14.81	47.01	
	5	10	53.8	3.94	6.26	9.41	12.59	15.98	49.89
1967	3	24	53.2	4.88	9.05	14.77	47.09		
	4	42	52.6	4.38	7.24	10.70	15.29	47.22	
	5	4	55.7	4.34	7.28	10.54	14.20	18.18	49.60
1966	4	30	52.3	4.52	7.05	10.79	15.08	47.45	
	5	5	52.9	4.65	7.52	11.97	15.78	19.59	49.78
1965	5	17	52.7	4.39	6.75	9.20	12.81	16.20	48.53

dahl 1969); it was inferred that an increase in male smolts would favor more grilse. Power (1973) suggested that many males may remain in the river as precocious parr to spawn escaping female grilse (Osterdahl 1969). This possibility was not examined in Western Arm Brook.

Large salmon were once present in this river (B. Sampson, Department Fisheries and Environment, Brig Bay, Newfoundland, personal communication) and now the spawning stock consists entirely of grilse. This could be the result of selection against larger sizes by the commercial fishery, perhaps evidenced by the 80% prevalence of net scarring on escaping grilse. The commercial fishery in St. Barbe Bay was monitored in 1975 and 1976, but the resulting estimates of 1889 and 2209 fish, respectively, were only approximate. The slight size-difference between grilse of different ages (Table 5) and the normal length-frequency distribution for grilse (Fig. 6) suggest that pressure of net selection would be equal for all ages. However, the sea survival rate for grilse that smoltified at 5 yr is only 3%, while it is 12% for grilse that smoltified at 3 yr and 6% for those at 4. Older smolt might remain at sea for 2 yr, which would increase the likelihood of their harvest in commercial nets as large salmon. If this is true and 3-yr-old smolts return as grilse, with relatively few being vulnerable to the fishery, then the 12% sea survival rate found for 3-yr smolt might be a realistic minimum survival rate prior to commercial exploitation. This agrees well with present estimates of 15% (Riche 1972).

Other studies found that the oldest smolts became grilse (Calderwood 1925; Hutton 1937), such that the total salmon age, i.e. smolt age plus sea age, remained constant (Gardner 1976). Ritter (1974) reported that the mean smolt age of grilse was higher than for salmon, but our calculations found his mean age differences nonsignificant ($P = 0.68$). The Department of Agriculture and Fisheries in Scotland (DAFS 1964) cited an increase in grilse survival with smolt age; the maximum sea survival rate in this report was 11.4% at smolt age 4 — equal to our findings at age 3.

There are data with trends compatible to ours. Dahl (1937) proposed that younger smolts were destined to be grilse and also suggested (Dahl 1916) that smolt age and sea age increased with northern latitude. Murray (1968) found that 3+ smolt had the greatest sea survival rate in Little Codroy River, Nfld. Sedgwick (1953) and Shearer (1966) have supported this view and proposed poorer feeding conditions as a reason for longer life histories in colder environments.

Ritter (1972) believed that age at maturity is influenced more by rate of growth in fresh water than by size at smoltification. As in our study, Sedgwick (1953) found that freshwater growth was greatest for younger smolts, and that the mean size at smoltification was similar regardless of smolt age. Many others have also shown that older smolts were slower growing (Menzies 1931; Jones 1959; Shearer 1966), and Piggins (1962) found artificially induced faster growth leads to younger age at smoltification. A factor that could contribute to slower growth of older smolt is sexual maturation of male parr (Mitans 1972).

We have indicated that these younger and faster growing smolts develop into grilse. Foerster (1968) found that sockeye salmon, *Oncorhynchus nerka*, which had greater sea age had also grown more slowly in fresh water. Shearer (1973) noticed that smolts from the same year-class that became grilse were larger than those that became salmon. Ritter (1974), on the contrary, found that faster growing and larger smolts were more likely to mature as salmon. Piggins (1974) also showed that younger smolts were more likely to become salmon. This has led to the practice of culling the

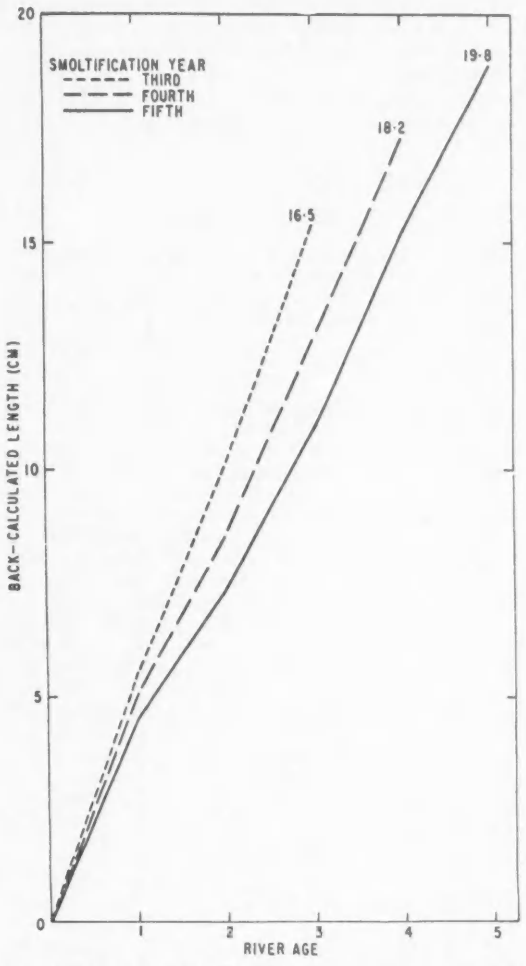

FIG. 3. Back-calculated lengths for the three types of smolts at all river ages (years). Mean size for each age at smoltification is shown at the top of the graph.

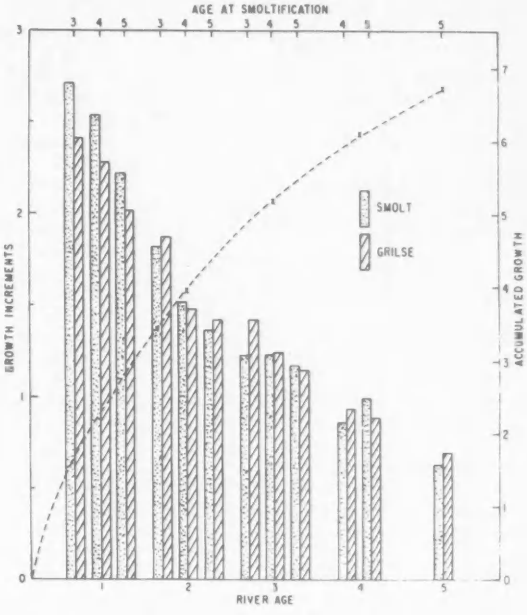

FIG. 4. Growth increments for smolts and grilse between each age at smoltification. Dashed curve shows accumulated growth for all fish combined.

smaller smolts from hatcheries (Peterson 1973), but before such practice is continued, more research should be performed.

Is the relationship between smolt age and duration of sea life attributable to genetic or environmental factors? Piggins (1974) demonstrated that smolts from salmon parents were more likely to become salmon. Elson (1973) also suggested that genetic factors were most important in determining sea age, although genetic factors cannot explain why most fish from these hatchery experiments returned as grilse, regardless of parentage. Furthermore, the data of Piggins (1974) and Elson (1973) show the best survival rates were from offspring of grilse parents. It is generally believed that larger rivers have a higher probability of producing large salmon (Calderwood 1949; Stewart 1949) and smaller rivers of producing grilse (Gardner 1976). Large rivers have more available space, which might allow for either genetic isolation (Gardner 1976) or perhaps a greater environmental gradient where, in some cases, there would be less nutrient in the upper reaches (Shearer 1966). Apparently, larger salmon migrate farther upstream (Berg 1959) and if this is true, their progeny would develop under less productive conditions and consequently exhibit slower growth rates and a greater age at smoltification. Any genetic influence could be explained by salmon and grilse parents spawning in different environments, which would allow for a manifestation of distinct growth rates. The profusion of opposing results beleaguering this field may be due to intrinsic variability in the selection of rivers studied to date.

The relationship between 1st-yr growth and year-class size was seen only when the different smolt ages were separated (Fig. 5). Parr may have been developing in different parts of the river system. The greatest 1st-yr growth was found in 3+ smolt. This age-group also had indications of density-dependent growth. Conceivably, these fish develop in parts of the river that are higher in nutrients but more restricted in terms of space. Concurrently, the almost linear increase in 1st-yr growth

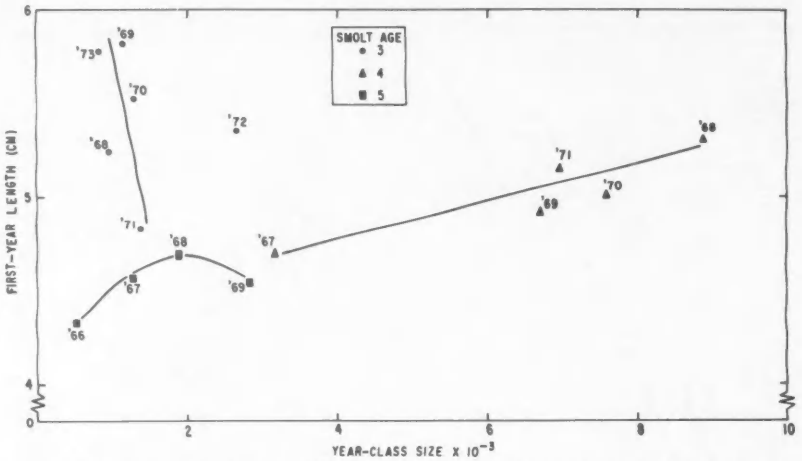

FIG. 5. Relationship between year-class size and back-calculated 1st-yr length for three smolt ages. All curves drawn by eye.

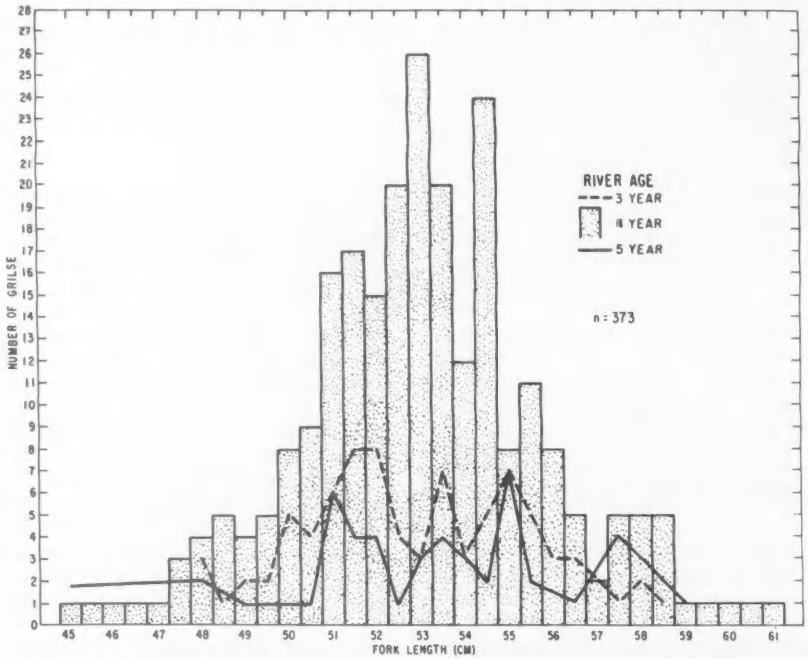

FIG. 6. Length-frequency distributions for grilse of three ages at smoltification counted in Western Arm Brook (1971–76).

with stock density among 4+ smolt might be indicative of low population densities, less competition, and a more moderate nutrient resource. Presumably if climatic effects were involved, growth rates would be consistent for all smolt ages in the same year-class, which they are not.

This study has quantified growth throughout the salmon's life history in a small Newfoundland river. Fish that smoltified at a younger age grew faster and had a greater chance of sea survival; most salmon smoltified at age 4+; fish that smoltified at older ages were of a slightly larger size; and grilse of different

smoltification ages had similar sea growth rates. We suggest local environmental differences within the watershed may have been responsible for the three types of smolt growth. Grilse may have the same genetic potential as large salmon. This suggests that a relaxing of the commercial fishery might result in a natural return of large salmon to the river.

Acknowledgments

We thank T. Anderson and B. Moores for efficiently supervising the field work; V. Pepper, D. Reddin and V. Taylor for reviewing the manuscript; T. Hutchings for typing; and Dr J. H. C. Pippy for his valuable comments and careful editing.

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Relation Between Temperature and Incubation Time for Eggs of Chinook Salmon (*Oncorhynchus tshawytscha*)

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ALDERDICE, D. F., AND F. P. J. VELSEN. 1978. Relation between temperature and incubation time for eggs of chinook salmon (*Oncorhynchus tshawytscha*). J. Fish. Res. Board Can. 35: 69–75.

With data assembled from the literature, relations are examined between incubation temperature and rate of development from fertilization to hatching for chinook salmon (*Oncorhynchus tshawytscha*) eggs. Ten forms of three empirical relations are used, based on the thermal sums hypothesis, Bělehrádek's equation, and a form of the logistic curve. In each case comparisons are made using constant, ambient, and combined (constant + ambient) incubation temperatures. In most cases the rules of minimum variance curve fitting are somewhat violated, although results using the log-inverse form of Bělehrádek's equation are superior to those from the other models. Early imposition of low, constant incubation temperatures (below 6–7°C) appears to slow egg development below those rates occurring at ambient temperatures having the same mean values. Time–temperature relations based on the unmodified thermal sums hypothesis and its associated "degree-days" are compared with predicted values obtained using the log-inverse Bělehrádek model as a standard. The former model tends to underestimate the length of the incubation period by an average of about 5 days at incubation temperatures above 4°C; below 4°C the former model increasingly overestimates the incubation period. Based on the log-inverse Bělehrádek model, a table is provided of predicted daily rates of development in relation to temperature. The table may be used to predict hatching time in a manner similar to that employing degree-days. In general, the enquiry points to a lack of data on development time at temperatures below 5°C; to be meaningful, further analyses will require such data.

Key words: prediction, modeling, development rates, hatching time

ALDERDICE, D. F., AND F. P. J. VELSEN. 1978. Relation between temperature and incubation time for eggs of chinook salmon (*Oncorhynchus tshawytscha*). J. Fish. Res. Board Can. 35: 69–75.

Les données extraites de la littérature nous ont servi à examiner les relations entre la temperature d'incubation et la vitesse de développement depuis la fécondation jusqu'à l'éclosion d'oeufs de saumon chinook (*Oncorhynchus tshawytscha*). Nous avons utilisé dix formes de trois relations empiriques fondées sur l'hypothèse des sommes thermiques, sur l'équation de Bělehrádek et sur une forme de courbe logistique. Les comparaisons sont faites dans chaque cas en utilisant des températures d'incubation constantes, ambiantes et combinées (constantes + ambiantes). Dans la plupart des cas, les règles de l'agencement des courbes avec variance minimale ont été quelque peu violées, quoique les résultats obtenus en utilisant la forme de l'inverse logarithmique de l'équation de Bělehrádek soient supérieurs à ceux des autres modèles. Des températures d'incubation constantes et basses (inférieures à 6–7°C) imposées au début semblent ralentir le développement de l'oeuf et lui imprimer des vitesses inférieures à celles de températures ambiantes de mêmes valeurs moyennes. Nous comparons les relations temps-température fondées sur l'hypothèse non modifée des sommes thermiques et des « jours-degrés » qui leur sont associés avec les valeurs prédites obtenues en utilisant le modèle de l'inverse logarithmique de Bělehrádek comme point de référence. Le premier modèle tend à sous-estimer la durée de l'incubation d'environ 5 jours en moyenne à des températures d'incubation supérieures à 4°C; à des températures inférieures à 4°C, le premier modèle surestime de plus en plus la durée de l'incubation. Utilisant le modèle de l'inverse logarithmique de Bělehrádek comme base, nous produisons une table des vitesses quotidiennes prédites de développement en relation avec la température. La table peut servir à prédire la date de l'éclosion de la même façon que la table fondée sur les jours-degrés. En général, l'étude fait ressortir le manque de données sur le temps de développement à des températures inférieures à 5°C; de telles données sont nécessaires à la poursuite d'études que l'on veut significatives.

Received August 3, 1976
Accepted September 1, 1977

Reçu le 3 août 1976
Accepté le 1 septembre 1977

Printed in Canada (J4497)
Imprimé au Canada (J4497)

THE influence of temperature on development of poikilotherms, and in particular on teleost eggs, has been much explored. The course of such enquiries has been summarized before (Embody 1934; Bělehrádek 1935; Fry 1947; Hayes 1949; Seymour 1956). Norris (1868) reported on experiments with eggs of brook trout (*Salvelinus fontinalis*) carried out by Ainsworth in 1859. Green (1870) concluded that trout eggs held at a mean temperature of 50°F (10°C) would hatch in 50 days, there being an increasing divergence from this one-to-one relationship at temperatures higher or lower than 50°F. Wallich (1901) concluded for chinook salmon (*Oncorhynchus tshawytscha*) eggs that the product of temperature (x) and incubation time (y) was constant at normal temperatures, that is:

$$xy = k. \tag{1}$$

He assumed that development would cease at the freezing point of fresh water. Reibisch (1902) and Apstein (1909) also investigated the relationship suggested by summation of thermal experience. They proposed the use of threshold temperatures as the lowest temperatures at which egg development would proceed. Threshold temperatures (biological zero), calculated from the distribution of rates of development at higher temperatures, tend to be slightly >0°C in fresh water. Another approach attempted to relate the velocity of development to earlier proposals of Van't Hoff and Arrhenius (Bělehrádek 1930) regarding changes in velocity of chemical reactions over a range of temperatures. Further modifications to the thermal sums hypothesis were suggested by Johnansen and Krogh (1914) and Bělehrádek (1935), and Janisch (1925) proposed the use of a catenary expression.

In terms of providing a simple relationship between temperature and length of incubation period, none of these empirical relations proves entirely satisfactory. Nevertheless, over the temperature range of 4.3–14.3°C, Seymour (1956) found that three methods gave reasonable approximations of the time–temperature relation. These were a modified thermal sums hypothesis

$$y = k/(x - c), \tag{1a}$$

Bělehrádek's equation (used here with the additional constant, c)

$$y = k/(x - c)^b, \tag{2a}$$

and a form of the logistic curve (Davidson 1944)

$$y = (1 + e^{a+bx})/k. \tag{3a}$$

In equations (1a) and (2a), y is development time, x is temperature, c is an estimate of the low temperature threshold for development, and k and b are constants. In the logistic expression (3a), k represents the distance between the upper and lower asymptotes of the sigmoid curve described by the equation, y and x representing time and temperature, as before.

Because of its simplicity in practical circumstances the thermal sums hypothesis, without consideration of the threshold temperature (i.e. $y = k/x$), has survived as a method for approximating the length of the incubation period. Nevertheless, Seymour (1956) concluded on the basis of all of his evidence that the logistic equation (3a) provided the best fit in relating the length of the incubation period to temperature, whether incubation temperature was constant or variable.

By reviewing factors affecting development and survival of Pacific salmon (*Oncorhynchus*) eggs, we undertook to gather currently available data relating temperature and the length of the incubation period. Two types of data were found: those where temperature was controlled and constant throughout incubation and those where temperature followed seasonal or ambient trends. Also, it quickly became evident that the limited available data would allow examination of the relation between incubation time and temperature for eggs of the chinook salmon (*O. tshawytscha*) only. For the other five species, including *O. masu*, the available data are insufficient, particularly for the pink salmon (*O. gorbuscha*).

There were two objectives in this enquiry: (a) using the available data and the models (equations 1a, 2a, 3a), to select that model best satisfying the rules of minimum variance curve fitting in describing the relation between development time or rate and temperature and (b) using the model selected, to tabulate the resulting rate–temperature relations for chinook eggs so that a more accurate means of determining hatching time could be obtained relative to estimates provided using the unmodified thermal sums hypothesis.

Methods

Incubation times for chinook eggs, found in the course of this survey, are presented in Table 1. Temperatures were considered "constant" if the range around a mean value was not >2°C. Data for egg development at ambient temperatures are based on the average of daily temperatures over the incubation period. In all instances the incubation period is taken as the interval in days between fertilization and 50% hatch of the eggs. Rate of development (percent per day) is equivalent to the fraction of the incubation period occurring daily at a given temperature. Temperature-associated rates of development to given stages of anatomical differentiation are not considered. It also is recognized that hatching may occur at different stages of differentiation and growth, depending among other factors on the conditions imposed during incubation; hatching therefore does not occur in all instances at a specific anatomical stage (Garside 1959). Nevertheless, prediction of hatching time is of practical significance to salmonid culturists, and accurate prediction remains a valid concern.

The general strategy followed was to obtain a fit to the data using the models, either in their standard (equation 1a–3a), inverse (equation 1b–3b), or logarithmic forms (equation 1c–3c) (see Table 2). In addition the logarithmic form of a transformation of the logistic expression (3b) was evaluated,

$$\ln([k - P]/P) = a + bx, \tag{3d}$$

where $P = 100/y$, a model considered by Seymour (1956)

TABLE 1. Development of chinook salmon eggs. Temperatures (constant; mean of ambient) associated with the incubation period (days) from fertilization to 50% hatch. Blocks of data are identified by source (see footnotes).

Temp (°C)	Period (days)	Temp (°C)	Period (days)	Temp (°C)	Period (days)	Temp (°C)	Period (days)	Temp (°C)	Period (days)
				Constant temperatures					
11.3[a]	44	4.5[b]	123.5	1.7	204.0	10.1	50.9	12.8	42
11.3	44	5.9	94.0	3.0	157.5	12.6	38.8	12.8	41
11.3	44	7.2	76.3	4.5	120.1	14.3	34.0	12.8	42
11.4	43	4.4	121.1	5.9	92.4	15.4	32.1	12.8	41
11.4	44	5.8	94.7	4.3[c]	128.6	16.7	30.7	12.8	42
11.4	43	7.1	78.9	7.1	79.1	13.1	38.0	11.3[e]	46.0
11.1	46	8.6	63.4	10.3	50.2	13.0	39.7	10.1[f]	50
11.1	46	9.6	55.4	12.8	40.0	12.8[d]	42	5.0[g]	110.0
11.1	46	9.9	52.2	15.7	34.0	12.8	41	11.0	47.3
7.2[b]	76.8	12.7	41.9	16.9	31.4	12.8	41		
1.6	206.3	14.2	36.7	18.1	28.0	12.8	42		
3.0	160.3	15.3	34.3	7.3	73.4	12.8	41		
				Ambient temperatures					
3.8[c]	132.0	15.0	32.7	14.4	36	6.0	82	5.7	87
4.3	112.0	13.3	40.3	15.2	36	6.0	84	5.7	87
9.7	56.0	13.2	43.4	14.0	37	5.9	84	5.7	86
13.3	38.7	12.7	43.3	14.5	35	5.9	84	5.7	85
16.3	31.9	10.7	50.3	15.0	35	5.9	84	5.8	87
3.8	133.0	9.9	57.8	15.6	36	6.0	87	5.8	86
4.8	102.0	2.8	144.0	16.4	33	5.9	88	5.9	87
9.1	62.2	2.5	158.0	14.0	36	5.8	87	5.9	86
12.9	42.3	2.3	172.0	14.5	35	5.8	87	5.9	85
16.3	33.5	13.0[d]	40	15.0	35	5.7	89	6.1	82
3.9	125.0	13.2	39	15.6	36	5.7	90	6.1	82
5.3	97.6	13.4	38	16.3	34	5.7	90	6.1	83
10.1	51.8	13.9	37	10.5[f]	48	5.7	89	6.1	81
13.5	38.1	13.0	39	10.4	48	5.6	89	6.1	84
3.9	124.0	13.2	39	10.3	49	5.6	88	6.1	81
5.3	97.4	13.5	37	10.1	50	5.6	88	6.3	80
10.3	48.6	14.0	35	10.0	50	5.6	88	8.9[h]	61
13.6	35.5	14.5	40	9.8	52	5.6	89	8.8	59
16.8	28.4	13.2	38	9.7	52	5.6	88	8.3	62
8.6	62.3	13.5	37	9.4	54	5.7	87	8.2	61
8.3	66.1	13.8	37	8.9	56	5.6	86	7.8	66
8.3	65.0	14.4	37	6.5	80	5.7	86	7.3	69
8.4	62.5	15.2	36	6.3	82	5.7	86		
14.7	32.4	13.2	39	6.1	81	5.7	85		
15.0	32.7	13.5	37	6.1	82	5.7	86		
15.0	32.7	13.9	36	6.1	83	5.7	87		

[a]Silver et al. (1963); [b]Burrows (unpublished data, see Seymour 1956); [c]Seymour (1956); [d]Donaldson (1955); [e]Griffioen (personal communication); [f]Wallich (1901); [g]Velsen and Alderdice (unpublished data); [h]Harvey (personal communication).

in rate–temperature studies of chinook egg development. For the three initial models, the resulting 10 forms were applied in turn to the constant, ambient, and pooled (constant + ambient) temperature data. The 10 sets of three comparisons obtained (total, 30) were evaluated as follows. In each case a measure was obtained estimating the closeness of fit of the observed points to the calculated regression line; hence

$$R^2 = (1 - \text{var}[y - \hat{y}])/\text{var}(y). \quad (4)$$

In addition, in each case a measure of mean-variance correlation was obtained, estimated from the relation between the residual variances and the predictor variables (i.e. x or $\ln x$). From this relationship, significant departures from homoscedasticity may be assessed, where

$$F = (r^2_{\text{res}}[n - k])/(1 - r^2_{\text{res}}), (\text{df} = 1, n - k), \quad (5)$$

n being the sample size and k the number of constants fitted in the expression employed.

A comparison also is made between the selected model and the unmodified thermal sums expression to assess the constancy of k in the latter expression. Finally, using the model selected, rates of development are tabulated over the useful temperature range.

Results

Computed coefficients for the 10 sets of prediction equations are listed in Table 2, along with their associated R^2 and r_{res} values. For all models a very high

TABLE 2. Minimum variance estimates of the parameters in expressions relating incubation time to 50% hatch (y) or rate of development ($P = 100/y$) and incubation temperature (x) in degrees Celsius for chinook salmon eggs. The models are based on (1) modified thermal sums hypothesis, (2) Bělehrádek's equation, and (3) forms of a logistic expression. Significance of mean-variance correlations may be assessed from the superscripts in the penultimate column, where for $|r_{res}|$: $^{0}\rightarrow P > .1$, $^{1}\rightarrow .1 > P > .01$, $^{2}\rightarrow .01 > P > .001$, $^{3}\rightarrow .001 > P$. The last column shows the calculated values of y at 10°C.

Equation and model[a]	Group (n)	$\hat{k}$	$\hat{a}$	$\hat{b}$	$\hat{c}$	R^2	r_{res}	y(10°C)
(1a) $y = k/(x - c)$	Constant (57)	625.068	—	—	−1.23778	0.97840	-0.62773^{3}	55.6
(modified thermal sums)	Ambient (126)	581.163	—	—	−0.98435	0.99008	-0.11712^{0}	52.9
	Pooled (183)	597.015	—	—	−1.10148	0.98292	-0.28460^{3}	53.8
(2a) $y = k/(x - c)^b$	C	22590.4	—	2.09994	−7.68048	0.99680	0.01292^{0}	54.2
(Bělehrádek)	A	1259.89	—	1.25654	−2.60027	0.99291	-0.21460^{1}	52.2
	P	1799.86	—	1.37077	−3.19011	0.98913	-0.48224^{3}	52.4
(3a) $y = (1 + e^{a+bx})/k$	C	0.03944	2.33787	−0.22655	—	0.99872	-0.03972^{0}	52.6
(inverse logistic)	A	0.03377	2.09818	−0.24663	—	0.99236	-0.23443^{2}	50.1
	P	0.03312	2.15660	−0.25716	—	0.98926	-0.47565^{3}	50.1
(1b) $P = (x - c)/k$	C	5.15797	—	—	0.04139	0.98946	-0.06883^{0}	51.8
(inverse modified thermal	A	5.34332	—	—	−0.39073	0.98643	0.59582^{3}	51.4
sums)	P	5.29074	—	—	−0.27305	0.98722	0.41733^{3}	51.5
(2b) $P = (x - c)^b/k$	C	7.89840	—	1.13239	−0.98189	0.99051	0.13376^{0}	52.4
(inverse Bělehrádek)	A	7.34983	—	1.09568	−1.25817	0.98663	0.63396^{3}	51.8
	P	7.30921	—	1.09800	−1.12572	0.98754	0.46600^{3}	51.9
(3b) $P = k/(1 + e^{a+bx})$	C	4.07973	2.31204	−0.21686	—	0.99126	0.64355^{3}	52.8
(logistic)	A	4.02197	2.08855	−0.20136	—	0.98671	0.52341^{3}	51.7
	P	4.07687	2.13913	−0.20271	—	0.98700	0.39689^{3}	52.0
(1c) $\ln P = \ln k + \ln (x - c)$	C	0.17998	—	—	−0.62994	0.98261	-0.66627^{3}	52.3
(log-inverse thermal sums)	A	0.18227	—	—	−0.61244	0.99086	0.27414^{2}	51.7
	P	0.18141	—	—	−0.62416	0.98772	-0.08661^{0}	51.9
(2c) $\ln P = \ln k + b \ln (x - c)$	C	0.03543	—	1.51053	−3.79690	0.99333	0.03274^{0}	53.6
(log-inverse Bělehrádek)	A	0.09968	—	1.18709	−2.07743	0.99231	0.36907^{3}	52.1
	P	0.08646	—	1.23473	−2.26721	0.99086	-0.03289^{0}	52.4
(3c) $\ln P = \ln k - \ln (1 + e^{a+bx})$	C	3.89849	2.32913	−0.22828	—	0.99651	0.52975^{3}	52.5
(log logistic)	A	3.69722	2.09317	−0.22185	—	0.99135	0.16103^{1}	50.9
	P	3.63489	2.15478	−0.23141	—	0.98979	-0.12780^{1}	51.0
(3d) $\ln ([k - P]/P) = a + bx$	C	(4.07973)	2.35068	−0.22049	—	0.99046	0.69887^{3}	52.9
(log-transformed logistic)	A	(4.02197)	2.10682	−0.20341	—	0.98158	0.48537^{3}	51.6
	P	(4.07687)	2.17236	−0.20604	—	0.98278	0.33503^{3}	52.0

[a]Fitting method used: all models except 1b, 3d — Gauss-Newton procedure; models 1b, 3d — linear least squares. In model 3d, the values of k obtained from model 3b were inserted prior to computation.

proportion of the total variance is associated with regression, and there is little opportunity to choose between the models on that basis (R^2, Table 2). There is some evidence of correlation between the residuals and predictor variables in each of the models investigated (r_{res}, Table 2). However, comparing all 10 models, heteroscedasticity appears to be minimized using the log form of the inverse Bělehrádek expression (equation 2c, Table 2); in this instance one would conclude for the pooled data that variance is homogeneous over the range of temperatures examined. Although there are only minor differences in length of the incubation period predicted from any of the models, at least at 10°C (Table 2), there is evidence of increased heterogeneity of variance in the remaining prediction equations. The log form of the transformed logistic (equation 3d) shows a high correlation between means and variances, as does the logistic expression (equation 3b). It is noted that transformation of equation 3b to 3d involves scaling equation 3b by a factor $1/k$; that is

$$P(1/k) = 1/(1 + e^{a+bx})$$

whence

$$k = P + P(e^{a+bx}),$$

$$(k - P)/P = e^{a+bx},$$

and

$$\text{(3d)} \qquad \ln ([k - P]/P) = a + bx,$$

a procedure that may be questioned on the basis that estimates of a and b obtained can be partly dependent on the value of k employed.

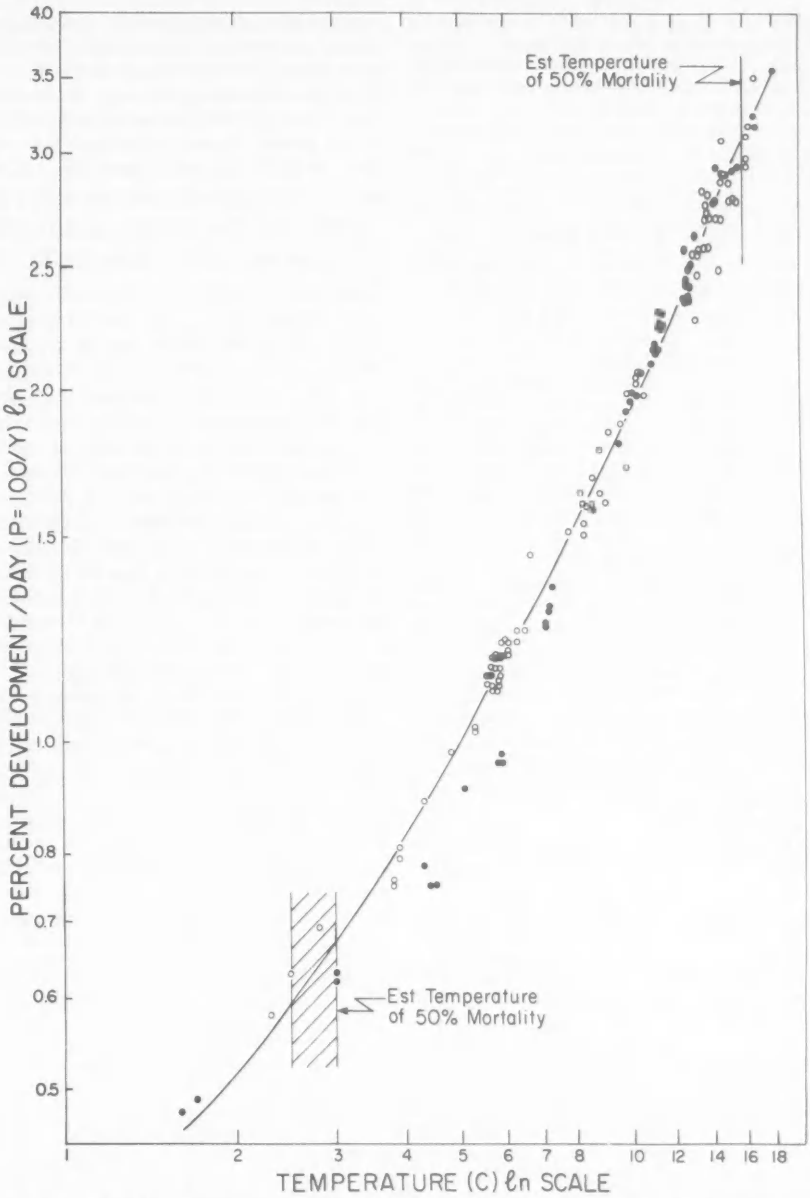

FIG. 1. Relation between percent development per day ($P = 100/y$; y = days) and temperature (x, °C) for chinook salmon eggs from fertilization to 50% hatch, using the pooled (constant and ambient temperature) data. The fitted line is obtained using the log-inverse Bělehrádek equation. The upper and lower incipient lethal temperatures for survival from fertilization to hatching are estimated from Seymour (1956) for constant temperatures. Temperature data: closed circles, constant; open circles, ambient temperatures of incubation.

Discussion

Given the models used and the data available, the relation between temperature and rate of development — as a daily percentage of the incubation period — is best described by equation 2c (Fig. 1). The choice remains provisional, however, as only 7 of the total of 183 data points are to be found in the lower third of the log-temperature scale in the figure, from about 1.6–

TABLE 3. Comparison of predicted rates of development ($1/y$, %/day) and incubation periods (y, days) at various temperatures using the log-inverse Bělehradek model (equation 2c) and the unmodified thermal sums hypothesis. Comparison is based on the pooled data.

Temp (°C)	Equation 2c[a]		Thermal sums[b]		Difference
	$1/y_1$ (%/day)	y_1 (days)	$1/y_2$ (%/day)	y_2 (days)	$(y_1 - y_2)$ (days)
1.0	0.373	268.1	0.213	468.7	−200.6
2.0	0.519	192.8	0.427	234.4	−41.6
3.0	0.673	148.6	0.640	156.2	−7.6
4.0	0.834	119.9	0.853	117.2	2.7
5.0	1.001	99.9	1.067	93.7	6.2
6.0	1.173	85.3	1.280	78.1	7.2
7.0	1.351	74.0	1.493	67.0	7.0
8.0	1.533	65.2	1.707	58.6	6.6
9.0	1.720	58.1	1.920	52.1	6.0
10.0	1.910	52.4	2.133	46.9	5.5
12.0	2.302	43.4	2.560	39.1	4.3
14.0	2.707	36.9	2.987	33.5	3.4
16.0	3.123	32.0	3.414	29.3	2.7
18.0	3.551	28.2	3.841	26.0	2.2

[a] $\ln 1/y = -2.44813 + (1.23473 \ln[x + 2.26721])$ (from Table 2).

[b] $y = 468.716/x$.

3.6°C. These few points could have a strong influence on the course of the fitted curve and on the choice of the model used. Obviously, more data are required at low temperatures, in general below 5°C, before further analysis would be meaningful.

The paucity of data at low temperatures notwithstanding, there is a suggestion from Fig. 1 of two different trends in developmental velocity below 6–7°C when rates are compared at constant and ambient temperatures. Developmental velocity seems somewhat slower at constant temperatures. Associated with this presumed difference is the time of establishment of thermal tolerance in the egg. Tolerance to low temperatures increases in some salmonid eggs once the initial period of early cell division is completed (Burrows 1963; Bailey and Evans 1971). Lower and upper lethal temperatures for chinook salmon eggs, those associated with 50% mortality from fertilization to 50% hatch, are about 2.5–3.0 and 16.0°C (Fig. 1). These limiting temperature levels are estimates obtained from egg incubation at constant temperatures (Seymour 1956). However, under natural incubation conditions winter minimum temperatures below 2.5–3.0°C occur normally, although at later stages of egg development. Hence, lower developmental velocity at low constant temperatures could be the result of unnatural low temperature stress during early cell division.

Accepting equation 2c as a provisional model, its ability to predict the length of the incubation period may be compared with that obtained using the unmodified thermal sums hypothesis ($y = k/x$), which still enjoys wide practical use. Using the Gauss–Newton procedure (Hartley 1961), the minimum variance estimate of k in the unmodified equation and for the pooled data (Table 1) is 468.716.

Using equation 2c as a standard for comparison, there is little difference in estimates of the incubation period for the two models at temperatures above 4°C. Above 4°C, the unmodified thermal sums hypothesis underestimates the length of the incubation period by an average of <5 days (Table 3). Near 3.5°C, the estimates essentially are equal. Below 4.0°C, however, estimates from the two models begin to diverge rapidly, the unmodified thermal sums hypothesis increasingly overestimating the length of the incubation period at

TABLE 4. Rate of development (%/day) for eggs of chinook salmon, based on the log-inverse Bělehrádek model[a] and using the pooled data (constant and ambient temperatures). Incubation period — fertilization to time of 50% hatch. Tabled values below the range of the available data (1.6–18.1°C) are extrapolated.

(°C)	0.0	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9
0	0.238	0.251	0.264	0.277	0.290	0.304	0.317	0.331	0.345	0.359
1	0.373	0.387	0.401	0.416	0.430	0.445	0.459	0.474	0.489	0.504
2	0.519	0.534	0.549	0.564	0.579	0.595	0.610	0.626	0.641	0.657
3	0.673	0.688	0.704	0.720	0.736	0.752	0.768	0.785	0.801	0.817
4	0.834	0.850	0.867	0.883	0.900	0.916	0.933	0.950	0.967	0.984
5	1.001	1.018	1.035	1.052	1.069	1.086	1.104	1.121	1.139	1.156
6	1.173	1.191	1.209	1.226	1.244	1.262	1.280	1.297	1.315	1.333
7	1.351	1.369	1.387	1.405	1.424	1.442	1.460	1.478	1.497	1.515
8	1.533	1.552	1.570	1.589	1.608	1.626	1.645	1.664	1.682	1.701
9	1.720	1.739	1.758	1.777	1.796	1.815	1.834	1.853	1.872	1.891
10	1.910	1.930	1.949	1.968	1.987	2.007	2.026	2.046	2.065	2.085
11	2.104	2.124	2.144	2.163	2.183	2.203	2.222	2.242	2.262	2.282
12	2.302	2.322	2.342	2.362	2.382	2.402	2.422	2.442	2.462	2.483
13	2.503	2.523	2.543	2.564	2.584	2.604	2.625	2.645	2.666	2.686
14	2.707	2.727	2.748	2.769	2.789	2.810	2.830	2.851	2.872	2.893
15	2.914	2.934	2.955	2.976	2.997	3.018	3.039	3.060	3.081	3.102

[a] Equation 2c: $\ln P = -2.44813 + (1.23473 \ln[x + 2.26721])$.

lower temperatures. Alternatively, a similar result is obtained by applying estimates of y, derived from equation 3c, to the unmodified thermal sums expression at selected temperatures. By this procedure, the value of k (degree-days from fertilization to 50% hatch) is maximized near 10°C, is reasonably constant between 4 and 18°C, but declines rapidly below about 4–5°C.

In summary, at incubation temperatures near and above 4°C, either the unmodified thermal sums hypothesis or the log-inverse Bělehrádek equation will provide reasonable estimates of the length of the incubation period. However, the latter model generally is more acceptable, particularly where low incubation temperatures occur.

An optional method of estimating incubation time is presented (Table 4), based on the combined data and the log-inverse Bělehrádek model. The tabled entries may be used in the same manner as degree-days are summed according to the thermal sums hypothesis; that is, daily predicted rates of development (percent per day), corresponding to the appropriate temperatures in the table, are summed over the incubation period. Fifty percent hatch should occur when the sum of the daily entries reaches 100.

Acknowledgments

The authors appreciate and acknowledge the aid of their colleagues: Mr J. Beukema, Dr J. Schnute, Ms Margot Alderdice (theory); and Mrs Arlene Sandnes, Messrs F. Nash and J. Thomson (computations). A substantial contribution to the paper was made by Dr D. P. Scott during review. We also thank Messrs R. Harvey and W. Griffioen for providing previously unpublished data on incubation times and temperatures for chinook salmon eggs.

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Seaward Migration of Hatchery-Reared Atlantic Salmon, *Salmo salar*, Smolts in the Penobscot River Estuary, Maine: Riverine Movements

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FRIED, S. M., J. D. MCCLEAVE, AND G. W. LABAR. 1978. Seaward migration of hatchery-reared Atlantic salmon, *Salmo salar*, smolts in the Penobscot River estuary, Maine: riverine movements. J. Fish. Res. Board Can. 35: 76–87.

Forty-six hatchery-reared Atlantic salmon, *Salmo salar*, smolts tagged internally with ultrasonic telemetry transmitters were tracked in the Penobscot River estuary, Maine. Travel times of six smolts tracked from head of tide 35 km seaward to the river mouth ranged from 29.4 to 44.5 h (mean 37.1 h). During 1975 and 1976 onset of increased movement of smolts occurred when hatchery and river water temperatures rose past 5°C. Full expression of migratory behavior occurred above 9°C. Earlier increased movement in 1976 coincided with earlier warming trends. Water current was the main factor influencing routes and rates of smolt movement. Smolts usually moved in the same direction as current flow during ebb and flood tides. This resulted in net seaward progress and fastest ground speeds (mean 56.4 cm/s) on ebb tide, and net upriver progress and slowest ground speeds (mean 37.4 cm/s) on flood tide. Swimming depth measured during portions of six tracks averaged 2.4 m. Simultaneous tracking of 1- and 3-m drogues together with smolts during five tracks showed that seaward progress of smolts was due mainly to passive drift. During times of current reversal or upon reaching shallow water, smolts often stopped moving, milled about, or apparently swam against the current. No meaningful correlations were found between travel speed and any environmental factors other than water current, except for influence of water temperature on initiation of migration. This study substantiates previous indications that smolt migration through the estuary is effected relatively quickly.

Key words: Atlantic salmon smolts, drift, estuaries, migration, telemetry

FRIED, S. M., J. D. MCCLEAVE, AND G. W. LABAR. 1978. Seaward migration of hatchery-reared Atlantic salmon, *Salmo salar*, smolts in the Penobscot River estuary, Maine: riverine movements. J. Fish. Res. Board Can. 35: 76–87.

Quarante-six smolts de saumon atlantique, *Salmo salar*, élevés en pisciculture et marqués à l'aide de transmetteurs télémétriques ultrasonores internes ont été suivis dans l'estuaire de la rivière Penobscot, Maine. Six smolts mirent 29.4–44.5 h (moyenne de 37.1 h) à parcourir une distance de 35 km depuis la tête des marées jusqu'à l'embouchure de la rivière. En 1975 et 1976, les smolts commencèrent à intensifier leurs mouvements au moment où la température de l'eau de l'écloserie et de la rivière dépassait 5°C. Le comportement migratoire s'exprime pleinement au-dessus de 9°C. Une augmentation hâtive des mouvements en 1976 coïncida avec des eaux qui se réchauffèrent plus tôt. Le courant est le facteur principal agissant sur les routes et les vitesses de migration des smolts. Les smolts se déplacent ordinairement dans la même direction que le courant, tant au jusant qu'au flot. Il en résulte un progrès net vers la mer et des vitesses-sol plus grandes (moyenne 56.4 cm/s) au jusant, et une progrès net en amont et des vitesses-sol plus petites (moyenne 37.4 cm/s) au flot. La profondeur moyenne de nage mesurée sur des portions de six trajets est de 2.4 m en moyenne. Le dépistage simultané de drogues à courant de 1- et 3-m et de smolts sur cinq trajets démontre que le progrès des smolts vers la mer est dû en grande partie à une dérive passive. En périodes de virement de courant ou en arrivant en eau peu profonde, les smolts s'arrêtent souvent, tournent en rond, ou semblent nager à contre-courant. Nous n'avons pas trouvé de corrélations significatives entre la vitesse de migration et aucun des facteurs de l'environnement autres que le courant, sauf

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Printed in Canada (J4837)
Imprimé au Canada (J4837)

pour ce qui est de l'influence de la température de l'eau sur le début de la migration. Cette étude corrobore les observations antérieures à l'effet que la migration des smolts dans l'estuaire s'accomplit avec une rapidité relative.

Received May 19, 1977
Accepted September 21, 1977

Reçu le 19 mai 1977
Accepté le 21 septembre 1977

ANADROMOUS Atlantic salmon, *Salmo salar*, once an abundant New England food and game fish, has been eliminated from all its native rivers of the U.S. east coast except for a few Maine rivers which support small populations (Netboy 1974; Baum 1971; Lennon 1976). The Penobscot River, Maine's largest river, was designated a model for Atlantic salmon restoration efforts by the Maine Sea-Run Salmon Commission and the U.S. Fish and Wildlife Service (Everhart and Cutting 1967). In 1975 and 1976, 190,000 smolts were stocked into the Penobscot River from two federal salmon hatcheries in Maine.

Little information is available on smolt migration patterns during estuarine and oceanic travel, and most theories on migration mechanisms have been based upon indirect evidence (Huntsman 1939; Harden Jones 1968; Stasko et al. 1973). Observations of smolts reaching the estuary suggest that schools may wander about for hours or days at the head of tide, sometimes swimming at the surface and jumping, especially during slack water (Calderwood 1908; Huntsman 1962; Stasko et al. 1973). A rapid trip through the estuary into the ocean was postulated based upon the "disappearance" of smolt schools with ebbing or spring tides (Calderwood 1908; Huntsman 1962), and recent tagging studies give further support to this theory (Bartel 1976).

This and a concurrent study (LaBar et al. 1978) form the first detailed account of smolt migratory behavior in an estuary. The specific objectives were to determine by ultrasonic telemetry (1) migration routes and rates of hatchery-reared Atlantic salmon smolts released into the Penobscot River estuary throughout the spring migration period, (2) the time within this period when fastest and most direct seaward travel occurred, (3) effects of time of day and stage of tide at release upon subsequent movement patterns, (4) effects of various environmental factors on speed and direction of travel, and (5) the extent to which movement was an active or passive event. This paper deals with movements of smolts in the upper, narrow, riverine portion of the Penobscot River estuary. The movements of these smolts in the lower, open-water portion of the estuary are reported elsewhere (LaBar et al. 1978).

Study Area

The portion of the Penobscot River estuary in which tracking was conducted flows in a narrow (mean width 1.0 km), relatively shallow (mean depth 9 m) basin from the head of tide release site in Brewer about 35 km seaward to Odom Ledge at the mouth of the river (Fig. 1). The most abrupt change in water depth occurs in the vicinity of Bucksport where channel depth increases from 8 m in the Frankfort Flats channel to 22 m in the Verona Island west channel (Fig. 1). Current velocity depends upon freshwater discharge and tidal current flow. Maximum downriver flow occurs during the last half of the ebb tide, while maximum upriver flow occurs during the last half of the flood tide. Several large, shallow coves (Smith Cove, Bald Hill Cove, South Orrington, Oak Point) (Fig. 1) trap water from the main current by causing the formation of eddies. Mean tidal range is 4.0 m at Bangor and 3.4 m at Bucksport.

Materials and Methods

TRACKING PROCEDURES

Ultrasonic tracking was conducted April to early June 1975 and 1976. A preliminary track done in June 1974 was also included in analyses.

Ultrasonic transmitters used were the smallest commercially available (9 mm diam × 33–36 mm long; 4–5 g in water) (Chipman Instruments, Madison, Wis.). Atlantic salmon smolts can carry internally placed transmitters of this size and weight without detrimental effects upon swimming stamina (McCleave and Stred 1975) or buoyancy regulation (Fried et al. 1976). Most transmitters were pulsed type (68–200 pulses/min; 45–59 kHz), but nine were continuous-wave transmitters (45–53 kHz).

In April 1975 and 1976, 1500 unmarked smolts at Craig Brook National Fish Hatchery, East Orland, Maine, were placed in a separate section of raceway for our use. To initiate a track, a group of smolts (usually 40) was netted and transferred to a transport tank in a truck. Either at the hatchery or at the river release site, the transmitter was activated, the receiving equipment tuned, one smolt was lightly anesthetized (100 mg/L solution MS-222®), the transmitter was pushed with a glass rod down the smolt's esophagus into the stomach, and the smolt's total length was measured. About 1 h was allowed between transmitter insertion and smolt release. All 40 smolts were placed in a holding net or box at the river release site just before liberation.

Releases throughout the season were made on four combinations of tide stage and time of day: high (ebb) tide/day, high tide/night, low (flood) tide/day, low tide/night. Tracks were started close to predicted times of high and low water. Day tracks were begun after sunrise and usually before noon, while night tracks were begun after sundown and usually before midnight.

Most tracks were initiated in fresh water near head of tide in a small cove shielded from the main current (Fig. 1). Some tracks were started near Winterport or Bucksport to ensure an adequate sample of smolts tracked into Penobscot Bay for a concurrent study (LaBar et al. 1978); the 1974 track was begun off Snub Point. Since these downriver release sites were within the saline portion of the river (Winterport releases, 0.2–4.7‰; Bucksport, 3.5–21.4‰), smolts to be released were first gradually acclimated over a 1-h period by successive additions of river water into the

TABLE 1. Tracking times, distances, and mean ground speeds of 46 Atlantic salmon smolts released into the Penobscot River estuary on various combinations of tide and time of day in 1974–76. Superscript for smolt number indicates reason for track termination. Smolt numbers without superscript denote smolts followed to the river mouth at Odom Ledge. BL/s = body lengths per second.

Smolt no.	Release				Smolt total length (cm)	Track duration (h)	Total travel			Net seaward distance (km)
	Date	Location	Time	Tide			Distance (km)	Mean ground speed (cm/s)	Mean ground speed (BL/s)	
					1974					
7401[d]	June 4	Snub Point	Day	Ebb	25.0	17.5	47.7	75.8	3.0	24.0
					1975					
7501[a]	April 8	S. Brewer	Day	Ebb	25.0	21.2	0.4	0.6	0.1	0.0
7502[a]	April 10	S. Brewer	Night	Flood	27.5	22.0	6.2	7.9	0.3	3.7
7503[a]	April 13	S. Brewer	Day	Flood	23.1	28.3	0.5	0.5	0.1	0.0
7504[c]	April 17	S. Brewer	Day	Flood	20.3	42.7	0.7	0.5	0.1	0.0
7505[a]	April 20	Winterport	Day	Flood	26.0	37.4	0.9	0.7	0.1	0.8
7506[c]	April 25	S. Brewer	Day	Ebb	21.6	48.0	0.6	0.4	0.1	0.4
7507[a]	April 28	S. Brewer	Day	Flood	21.6	48.5	1.7	1.0	0.1	0.0
7508[b]	May 3	S. Brewer	Day	Ebb	22.0	69.0	1.2	0.5	0.1	0.8
7509[b]	May 6	S. Brewer	Night	Flood	18.5	19.0	1.6	2.3	0.1	1.4
7511[b]	May 8	S. Brewer	Night	Ebb	23.5	3.8	1.7	13.0	0.6	1.6
7512	May 10	S. Brewer	Night	Flood	23.0	44.5	70.9	44.3	1.9	34.5
7513[a]	May 13	S. Brewer	Night	Flood	21.5	25.3	27.3	15.4	0.7	16.3
7514[a]	May 14	S. Brewer	Day	Flood	27.5	1.3	2.7	55.5	2.0	2.6
7515[b]	May 15	S. Brewer	Day	Flood	25.7	6.0	4.0	18.4	0.7	3.7
7516[a]	May 16	S. Brewer	Night	Flood	25.7	14.8	20.6	38.9	1.6	17.5
7517[b]	May 20	Winterport	Night	Ebb	22.7	5.7	12.6	61.8	2.7	11.6
7518	May 21	S. Brewer	Day	Ebb	23.7	29.4	53.1	50.2	2.1	34.5
7520	May 24	Winterport	Night	Flood	23.5	20.8	41.8	55.9	2.4	17.1
7521	May 27	S. Brewer	Night	Flood	22.5	33.7	51.8	42.7	1.9	34.5
7523	June 2	S. Brewer	Night	Ebb	22.5	40.6	59.2	40.5	1.8	34.5
7524[a]	June 6	S. Brewer	Day	Ebb	22.5	11.3	11.2	27.4	1.2	6.9
1975 Means					23.3	27.3	17.7	18.0	0.8	10.6
					1976					
7601[e]	April 14	S. Brewer	Day	Ebb	23.0	41.8	1.5	1.0	0.1	1.2
7602[e]	April 22	S. Brewer	Day	Flood	22.0	41.7	0.4	0.3	0.1	0.1
7603[e]	April 28	S. Brewer	Day	Ebb	24.0	51.7	19.1	10.3	0.4	11.8
7604[a]	May 2	S. Brewer	Night	Flood	24.0	3.0	0.8	7.7	0.3	0.8
7605[a]	May 3	S. Brewer	Day	Flood	23.0	2.6	5.9	61.5	2.7	5.7
7606[e]	May 4	S. Brewer	Day	Flood	25.0	26.0	11.6	12.2	0.5	9.8
7607[e]	May 7	S. Brewer	Day	Ebb	25.0	22.7	2.0	2.4	0.1	1.6
7608[e]	May 8	S. Brewer	Day	Ebb	23.1	29.7	29.9	28.0	1.2	3.7
7609[b]	May 10	S. Brewer	Day	Ebb	23.0	28.6	30.4	29.5	1.3	20.2
7610[b]	May 12	Winterport	Day	Ebb	24.5	1.0	1.5	40.7	1.7	1.4
7611[a]	May 13	S. Brewer	Day	Ebb	27.0	3.7	8.6	65.1	2.4	8.1
7613[a]	May 14	S. Brewer	Day	Ebb	29.0	47.6	49.2	28.7	1.0	13.6
7614	May 17	S. Brewer	Night	Flood	28.5	34.8	57.1	45.6	1.6	34.5
7615[b]	May 20	Winterport	Day	Ebb	22.2	12.7	21.1	46.3	2.1	6.9
7616[a]	May 20	S. Brewer	Day	Flood	25.0	31.7	15.3	13.4	0.5	8.3
7618[a]	May 24	Bucksport	Night	Ebb	25.0	1.3	4.3	90.3	3.6	4.1
7619[e]	May 25	S. Brewer	Day	Ebb	24.0	11.9	19.2	44.5	1.9	14.4
7620	May 25	Bucksport	Night	Flood	25.2	2.0	8.8	104.7	4.2	8.5
7621	May 26	Bucksport	Night	Ebb	25.4	6.0	10.7	49.4	1.9	8.7
7622[e]	May 28	S. Brewer	Day	Ebb	24.0	28.0	9.7	9.6	0.4	9.3
7623[e]	May 30	Bucksport	Night	Ebb	22.3	18.3	3.0	4.5	0.2	2.0
7624	June 1	Bucksport	Day	Ebb	26.3	14.3	19.9	38.6	1.5	12.6
7625	June 6	Bucksport	Day	Ebb	25.0	3.0	8.7	80.2	3.2	8.3
7626[a]	June 8	S. Brewer	Day	Ebb	25.0	2.8	0.6	6.1	0.2	0.2
1976 Means					24.6	19.5	16.2	23.2	0.9	8.2

[a]Tag failure. [b]Lost signal. [c]Lack of movement. [d]Crew fatigue. [e]Hydrophone malfunction.

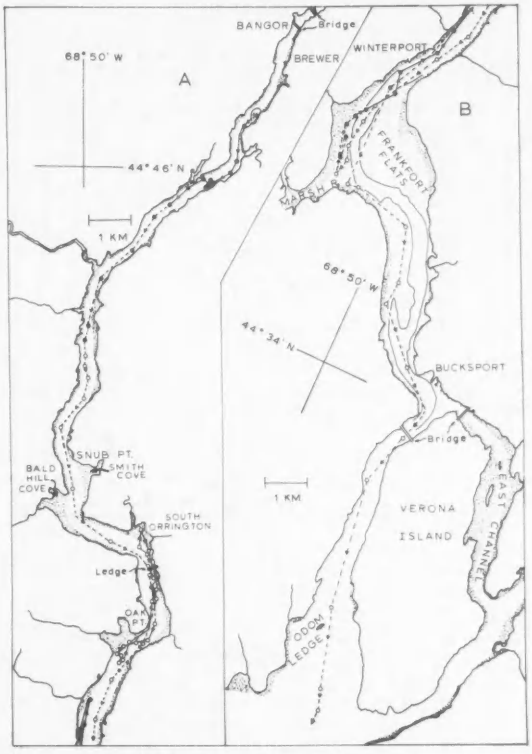

FIG. 1. Penobscot River estuary, Maine, showing route followed by Atlantic salmon smolt 7614 during ultrasonic tracking in May 1976. Each circle represents a position plot, usually every 20 min. Open circle = day, closed circle = night, solid line = flood tide, dashed line = ebb tide. A, The upper, mostly freshwater portion of the study area; B, the lower, saline portion of the study area. Intertidal areas are stippled. Main river channel denoted by solid lines only in Frankfort Flats area (B).

transport tank. No mortalities occurred and smolts never exhibited obvious signs of stress.

Two tracking vessels were used, a 5-m Boston whaler and an 11-m diesel inboard vessel. Both were equipped with similar receiving devices and rotatable, directional hydrophones. The hydrophone in the 11-m vessel was mounted in a streamlined plastic pod attached to a faired housing to reduce drag and water noise (Stasko and Polar 1973).

During tracking, the boat either was allowed to drift or was moved toward the smolt on bearings obtained from the hydrophone. Smolt position was plotted every 20 min on navigation charts after the boat was moved close to the smolt. Positions in the narrower portions of the river were determined by eye, using charted landmarks in conjunction with water depth. A sextant was used in more open areas and the 11-m vessel also used radar whenever necessary. A total of 56 swimming-depth measurements was obtained during portions of tracks 7506, 7507, 7513, 7523, 7624, and 7625 by use of a linear-array hydrophone (Gardella and Stasko 1974).

Whenever possible where the fish's position was determined, measurements were made of current velocity, water depth, water temperature, salinity, wind velocity, and wave direction. Cloud cover, sun or moon visibility, precipitation, and wave height were estimated. Barometric pressure was continuously recorded at Orono, Maine. Water temperature, salinity, and current velocity were measured at 1- and 2-m depths from the 5- and 11-m vessels, respectively.

Additional data on water currents were obtained during 1976 with current drogues constructed from two metal vanes (46 × 46 cm) fastened at right angles to each other and suspended at the desired depth from small floats. Daytime releases of drogues from head of tide were made during ebb tide on May 13 (five 1-m and five 3-m drogues) and again on June 2 (six 1-m and six 5-m drogues). In May drogue positions were plotted once or twice before nightfall and once the following morning. In June drogue positions were plotted 3 or 4 times during a 6-h period. Simultaneous tracking of 1- and 3-m drogues along with tracking of a smolt was conducted irregularly throughout the season.

TRACKING RECORD

Forty-six Atlantic salmon smolts (mean total length 24.0 cm) released into the Penobscot River estuary were tracked a total distance of 809 km for a total time of 1059 h. Individual tracks covered total distances of 0.4–70.9 km (mean 17.7 km, 1975; 16.2 km, 1976) and lasted 1.0–69.0 h (mean 27.3 h, 1975; 19.5 h, 1976) (Table 1).

Five smolts (7512, 7518, 7521, 7523, 7614) were followed continuously from head of tide to Odom Ledge (Table 1; Fig. 1). One smolt (7619) was initially tracked only 14.4 km seaward from head of tide, but was relocated 27 h later about 16 km farther downriver in the Verona Island west channel, and followed to Odom Ledge. Five other smolts were tracked to Odom Ledge from release points at Snub Point (7401), Winterport (7520), and Bucksport (7620, 7621, 7624). Of the remaining tracks, 25 were terminated due to transmitter failure or signal loss and 10 were abandoned when smolts failed to make significant seaward progress after at least 10 h (Table 1).

DATA ANALYSIS

To determine actual smolt swimming velocities, corrections must be made for water current velocities, since the direction and speed of movement of a smolt relative to the ground is the vector sum of movement of the smolt and movement of the water. During tracks frequent current velocity measurements were made using current meters. Unfortunately current speeds measured with current meters unpredictably underestimated current speeds as measured with drift drogues, although meters and drogues were in good agreement when measuring current direction. Since only limited drogue tracking was done, all results on migration rates are presented as over-the-ground travel rather than actual movement through the water.

Calculations of smolt travel directions, distances, and ground speeds were based on straight lines between successive position plots. Data for each smolt were grouped by stage of tide (ebb and flood) and time of day (day and night). For each smolt the mean of all ebb tide ground speeds was compared with the mean of all flood tide ground speeds by a *t*-test. Similar comparisons were made between day and night ground speeds during ebb tide and

between day and night ground speeds during flood tide. Plots during which smolts were not moving were not considered. Mean hourly ground speeds and net hourly seaward progress were calculated in relation to stage of tide and time of day to determine whether tidal or diel periodicities occurred in smolt activity; all plots were considered for these calculations.

Smolt bearings between successive position plots were also calculated relative to water current direction; for each smolt mean travel bearings relative to water movement (i.e. empirical mean vectors) (Batschelet 1965) were computed separately on day ebb, night ebb, and total ebb tide plots and on day flood, night flood, and total flood tide plots. A modification of Rayleigh's test was used to test the hypotheses that smolts traveled in the same direction as the water current or in the opposite direction to the current, and Rao's test was used to test for the occurrence of more than one clustered direction relative to water current within any array of smolt bearings (Batschelet 1972).

Correlation coefficients were calculated between smolt ground speeds and all measured or estimated environmental factors.

Results

Seasonal Timing of Migration

Initiation of seaward migration both years followed a period of rising hatchery water temperatures. An earlier increase in movement of smolts tracked during 1976 corresponded to an earlier increase of hatchery water temperature to above 5°C (April 24, 1976; April 30, 1975) (Fig. 2). Similar warming trends occurred at river release sites, and river water temperatures at release times of tracks started prior to and including May 10 never differed from hatchery water temperatures by more than 2.5°C (mean 0.7°C, 1975; 0.9°C, 1976). After May 10 river temperatures always exceeded hatchery temperatures by 1.2–8.9°C at head of tide and Winterport release sites (mean 5.7°C, 1975; 3.4°C, 1976), but were as much as 1.3°C colder at Bucksport (mean 0.6°C, 1976) (Fig. 2). Differences in smolt movement patterns could not be related to differences between water temperatures at release sites and those at the hatchery. Initiation of migration corresponded to times when river and hatchery temperatures approached or exceeded about 9°C (Fig. 2). However, although water temperatures warmed more quickly in 1976, real seaward migration began about May 10 both years (Table 1; Fig. 2). Once smolts were migrating their movements were unrelated to water temperature, and they continued uninterrupted even at temperatures as low as 6°C in lower sections of the estuary.

Although the field season during both years could be clearly divided into a premigratory phase during which smolts moved only sporadically, usually remaining near the release site, and a migratory phase during which smolts consistently made seaward progress, greater variation in individual migratory behavior was noted in 1976.

In 1975 smolts released prior to May 8 generally

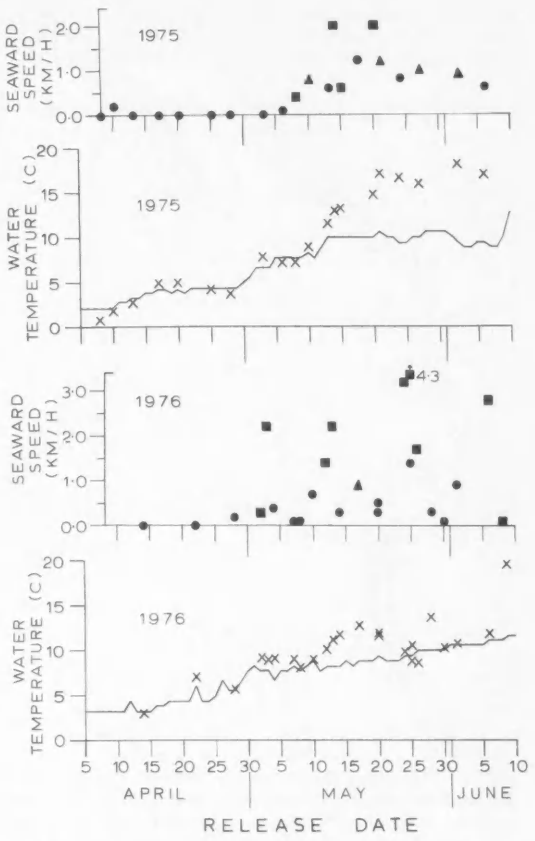

Fig. 2. Mean seaward travel speeds of smolts, river water temperature at times of release, and mean daily hatchery water temperature during ultrasonic tracking studies. Circle = smolt tracked more than 6 h, square = smolt tracked 6 h or less, triangle = smolt tracked entire length of study area, cross = river temperature at time of release, solid line = mean daily hatchery water temperature. Temperature reading not taken at time of release on May 16, 1975 (7516).

did not move much. Greatest seaward travel for a smolt tracked during this period was only 3.7 km in 22 h (smolt number 7502, Table 1). The first smolt (7511), which immediately left the release site to travel quickly seaward, was released May 8, but was lost after only 3.8 h. The first long seaward track (7512) was made May 10. All smolts released after this date made regular seaward progress (Table 1), and there were few periods when movement of these smolts ceased.

In 1976 most smolts tracked prior to May 8 left the release site. While three of these smolts made more than 3 km net seaward progress, two (7603, 7607) were eventually abandoned due to lack of movement, and the third (7605) was only tracked a short time (2.6 h) before its transmitter failed (Table 1). Although the

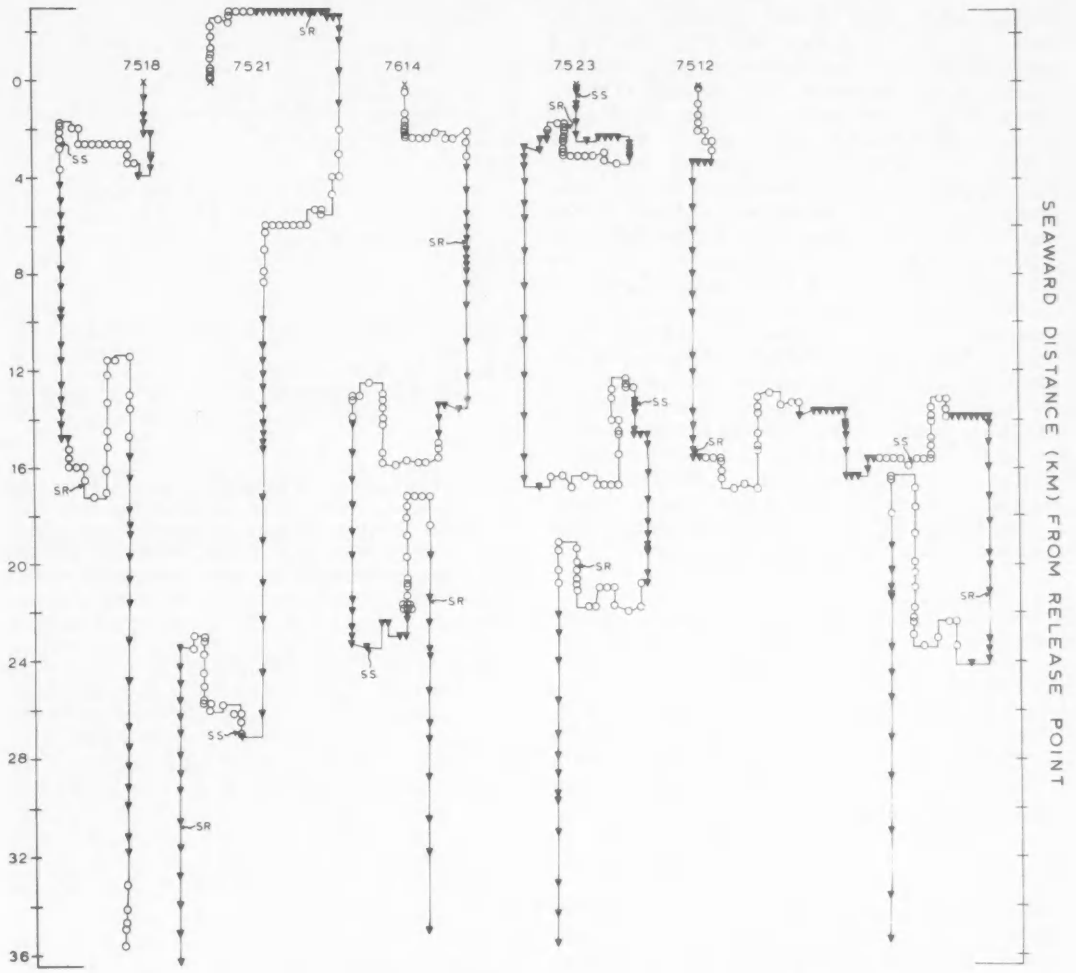

FIG. 3. Schematic representations of tracks of five smolts followed at least 35 km seaward from head of tide. Symbols represent position plots at 20-min intervals. Distance between plots denotes actual distance traveled up- or downriver. Rows of symbols perpendicular to distance axis denote periods when smolts were not moving. Starting point of each track is at top. Cross = release point, open circle = flood tide, triangle = ebb tide, SS = sunset, SR = sunrise.

smolt released May 8 (7608) moved a considerable total distance (29.9 km), it mostly wandered in shallow water and traveled only 3.7 km seaward in 29.7 h before it was finally abandoned. The first long seaward track (7609) was again made May 10, but not all smolts released after this date made regular seaward progress. Two smolts, although ultimately abandoned due to transmitter failure, traveled only 8.3 km (7616) and 13.6 km (7613) seaward from head of tide in 31.7 and 47.6 h, respectively. Two other smolts were abandoned due to lack of movement. One of these (7622) made only 8.0 km net seaward progress in the first 18 h after release at head of tide and moved little during the remaining 10 h before the track was ended. The other (7623), released above Bucksport, entered Bucksport harbor and remained there for 16 h until tracking was terminated.

MIGRATION ROUTES

All five smolts tracked continuously from head of tide to Odom Ledge traveled much farther (mean 58.4 km) than the shortest distance between the two points (34.5 km) (Fig. 3). Departures from the shortest route

resulted mainly from upriver movement within the main river basin during flood tides (Fig. 1 and 3). A choice of alternative seaward routes occurred where the estuary was bisected by Verona Island (Fig. 1). Eleven of 12 smolts tracked past this choice point traveled down the shorter, deeper (main) west channel.

While smolt movements were generally restricted to the main channel by the narrow width of the river basin, smolts often traveled close to shore and over shallow intertidal areas. Only three smolts entered any of the large tidal coves. Two of these entered Bald Hill Cove and milled about for 4.3 h (7603, 0.5 km) and 5.0 h (7613, 1.6 km). The third (7614) moved briefly towards the head of the cove at South Orrington (0.2 km) (Fig. 1). Both smolts entering Bald Hill Cove were later abandoned due to lack of movement. None went up any of the tributaries flowing into the estuary. The exact route taken through the Frankfort Flats area, the widest portion of the study area (Fig. 1), depended upon tide stage and current velocity. Routes varied from direct seaward travel, mostly in the narrow, main channel, to considerable up- and downriver movement, often over the tidal flats and sometimes close to shore.

Migration Rates

Mean ground speeds of smolts released prior to May 8 were generally much lower (mean 1.1 cm/s, 1975; 6.1 cm/s, 1976) than speeds of smolts released later in the season (mean 41.8 cm/s, 1975; 29.8 cm/s, 1976) (Table 1). Mean net seaward progress of smolts during the migratory phase of the season was 0.9 km/h in 1975 and 0.6 km/h in 1976. Travel times of smolts tracked from head of tide to Odom Ledge (including 7619) ranged from 29.4 to 44.5 h (mean 37.1 h). No relationship was found between mean ground speed and size of smolts.

Comparisons between ebb and flood tide ground speeds for each smolt showed that most smolts released on and after May 8 moved significantly faster on ebb tide (overall mean 56.4 cm/s, ebb; 37.4 cm/s, flood) ($P < 0.01$, t-test). Six smolts active before May 8 generally moved at similar, slow speeds during ebb and flood tides (overall mean 23.2 cm/s, ebb; 27.6 cm/s, flood). No significant differences between ground speeds during ebb and flood tides were found for five of these smolts ($P > 0.05$, t-test), but the sixth (7606) traveled significantly faster during flood tide (mean 12.5 cm/s, ebb; 42.5 cm/s, flood) ($P < 0.01$, t-test).

Significant differences were found between smolt ground speeds during day and night portions of ebb tide and also during day and night portions of flood tide for some smolts ($P < 0.01$, t-test). This was due only to the area of the estuary a smolt was moving through during day and night portions of a track. Fastest currents, resulting in fastest smolt ground speeds, occurred in the more seaward portions of the estuary. Smolts migrated day and night; no diel periodicities in travel rates were found.

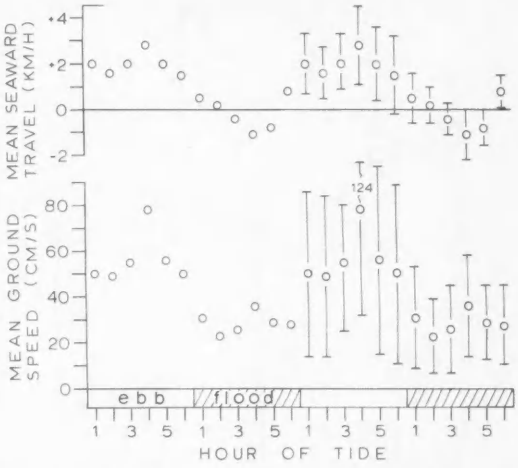

FIG. 4. Tidal pattern of mean hourly seaward transport and mean hourly ground speeds computed from data from five smolts carrying ultrasonic transmitters and tracked 35 km seaward from head of tide. Means for ebb and flood tides plotted twice to show trends more clearly. Bars = ±1 SD.

Mean hourly travel rates of the five smolts tracked the entire length of the estuary followed a definite pattern in relation to tide stage. Net seaward progress and fastest ground speeds occurred during ebb tide, while net landward (upriver) progress and slowest ground speeds occurred during flood tide (Fig. 4). These smolts spent a slightly larger proportion of time traveling during ebb tide portions of tracks (mean 84% ebb; 77% flood), but this was not great enough to account for observed seaward progress differences on ebb and flood tides.

Swimming Depth

During portions of six tracks for which swimming depth measurements were obtained, smolts traveled at a mean depth of 2.4 m and a mean distance of 7 m above the bottom. Smolts were at or near the bottom during only five measurements; at these times smolts were out of the main channel and had either stopped moving or were moving slowly. No differences were noted in swimming depth between ebb and flood tide ($n = 36$ ebb, 20 flood) or between day and night ($n = 33$ day, 23 night).

The most complete series of measurements ($n = 31$) was made during a 14.3-h track (7624). This smolt, released at Bucksport, traveled in the top 3 m of the water column well above the river bottom most of the time (Fig. 5). However, it was on or near the bottom for at least 1 h early in the track when it left the main channel and milled about in the relatively shallow water (5 m) at the entrance to the Verona Island east chan-

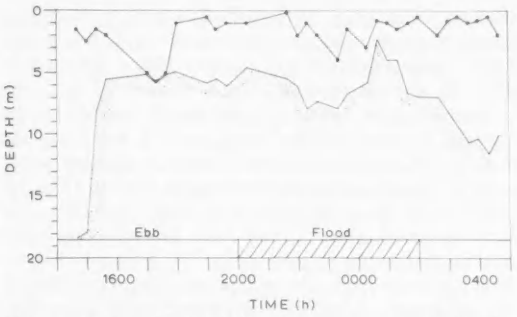

FIG. 5. Swimming depth in relation to water depth, time of day, and tide stage of smolt 7624 tracked from Bucksport into the east channel around Verona Island. Each point on the upper curve represents a swimming depth measurement. Water depth is indicated by the lower curve.

nel. This was the only smolt to travel down the east channel around Verona Island.

RESPONSE TO WATER CURRENT

The importance of water current in determining overall patterns of smolt movement was clearly shown by plotted courses of smolts (Fig. 1 and 3). Down- and upriver travel generally coincided with ebb and flood tides, respectively, while sudden changes in travel direction often closely approximated times of high and low water (i.e. times of changing current velocities).

Travel directions of smolts between successive position plots were clustered in the direction of water current flow during ebb or flood tide for 18 of 19 smolts tracked for 12 h or more on and after May 8 (Fig. 6A). Of these 18 smolts, 10 traveled in the current direction during ebb and flood tides, 7 during ebb tide only, and 1 during flood tide only ($P < 0.05$, modified Rayleigh's test). Only one of the smolts that traveled consistently with the current during ebb tide only was continuously tracked the entire length of the study area. This smolt (7521) moved upriver from the head of tide release site during flood tide (Fig. 3), while current direction measured 1 m below the surface was downriver. The smolt that traveled consistently with the current during flood tide only (7622) and the smolt that did not travel consistently with the current during either ebb or flood tide (7623) made net downriver progress, but both were later abandoned due to lack of movement.

Only four smolts released prior to May 8 moved in a consistent direction in relation to current flow. One smolt traveled in the same direction as water current during ebb and flood tides, two only during ebb tide, and one only during flood tide ($P < 0.05$) (Fig. 6B).

No significant clustering of smolt directions counter to current direction ($P > 0.05$) and no significant biomodal response ($P > 0.05$, Rao's test) was found for any smolt during either ebb or flood tide.

Ebb tide releases of 1-, 3-, and 5-m current drogues at head of tide indicated that observed smolt travel rates

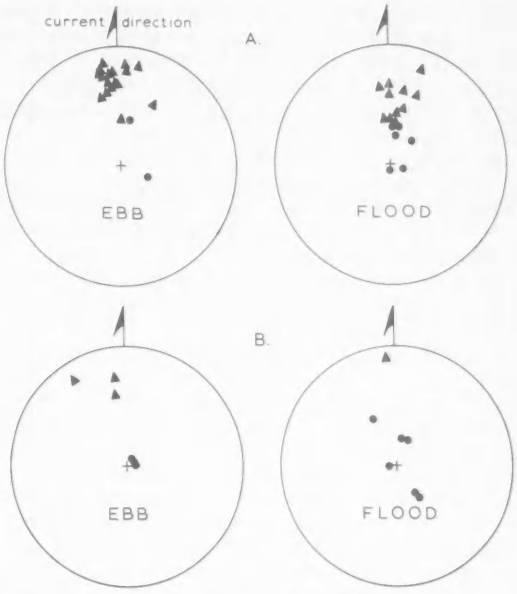

FIG. 6. Mean direction of smolt movement in relation to ebb and flood tide water current flow direction measured 1–2 m below the surface. Each symbol represents an empirical mean vector computed from all ebb or all flood tide travel directions measured between successive position plots, usually 20 min, during ultrasonic tracking of one salmon smolt. Distance of each symbol from center of circular diagram denotes length of mean vector. Triangle = mean vector significant ($P < 0.05$, modified Rayleigh's test), circle = mean vector nonsignificant. A, Smolts released May 8 through early June; B, smolts released early to mid-April up to May 8.

could result from passive drift. Fastest times for smolts to travel 11.5 km seaward from head of tide (3.5 h, 91.3 cm/s, 7619; 3.6 h, 88.7 cm/s, 7613) were in close agreement with drift times of drogues (mean 3.4–4.9 h, 93.0–65.3 cm/s). Both these smolts were released during ebb tide and traveled in the main channel. Smolts taking longer times to traverse this section of estuary were either released on flood tide (7.3–19.1 h) when current speeds were low and current directions variable, or were released on ebb tide but moved slowly or delayed moving and encountered flood tide before traveling 11.5 km seaward (7.7–17.3 h). Many current drogues became grounded in shallow areas nearshore either shortly after release or several hours later, indicating that water currents could carry smolts out of the main channel into shallow water, thus delaying seaward progress. Only two drogues (5 m) were recovered drifting freely after being released over 24 h earlier at head of tide. Net seaward travel rates of both these drogues (0.9 km/h) were in good agreement with those calculated for migrating smolts (mean 0.9 km/h, 1975; 0.6 km/h, 1976).

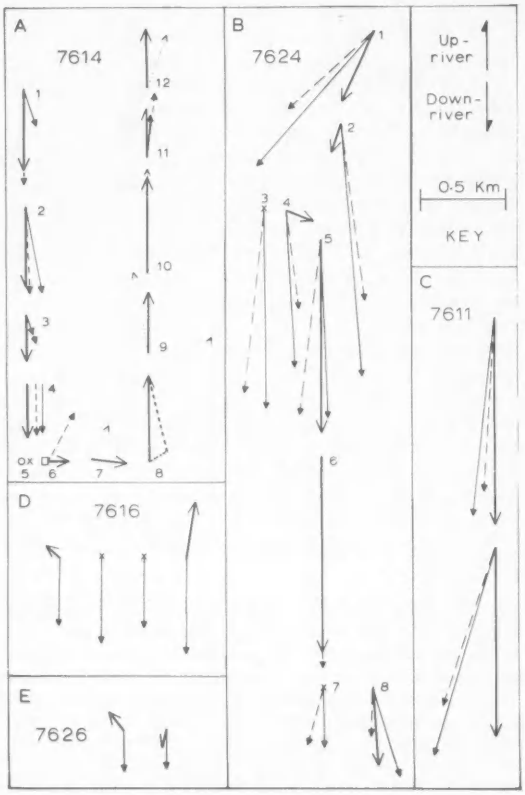

FIG. 7. Schematic representation of transport vectors of five smolts tracked simultaneously with 1- and 3-m current drift drogues. Vectors in A and B arranged to show movement of smolts during successive position plots and numbered consecutively. All transport vectors are for 20-min intervals except for two vectors at point 8 in A. Thick arrow with open head = smolt vector, thin arrow with solid head = 1-m drogue, dashed arrow with solid head = 3-m drogue, dotted arrow with open head = 1- and 3-m drogues (i.e. similar movement), cross = smolt not moving, circle = 1- and 3-m drogues not moving, square = 1-m drogue not moving. A, Smolt tracked as water current direction reversed during flood tide. Drogue and smolt vectors at point 8 are for a 40-min interval (two 20-min smolt vectors are indicated by heavy dashed lines); B, smolt tracked from main channel at Bucksport to entrance of east channel around Verona Island (points 1–4) and into east channel (points 5–8); C, smolt tracked in main channel; D and E, smolts tracked in shallow water, close to shore.

The most convincing evidence that smolt migration through the estuary is largely passive was provided by simultaneous tracking of 1- and 3-m drogues together with each of five smolts (7611, 7614, 7616, 7624, and 7626) for periods from 0.7 to 4.7 h. Velocities of both drogues usually closely approximated velocities of smolts (Fig. 7A, B, C), except when smolts were in shallow water (Fig. 7B [points 2–4 and 7], D, E) or during times of tidal current reversals (Fig. 7A, points 5–8). Depth measurements made during track 7624 (Fig. 5) showed that this smolt was on or near the bottom when its movements differed from that of the drogues, but was drifting in the surface waters during most of the drogue tracking. Smolts in shallow water or close to shore often either were not moving (Fig. 7B [point 3], D, E) or moved against the current (Fig. 7D, E). One smolt (7614) may have moved against the current as the tide turned (Fig. 7A, points 5–8), although it was eventually carried into the main channel and upstream as current speed increased (Fig. 7A, points 8–12). Only during two plots did smolts seem to travel faster than drogues (Fig. 7B, C, points 5 and 1, respectively), but differences were slight and could have been due solely to errors inherent in position determination.

Response to Other Environmental Factors

No other environmental factor appeared to influence smolt movements, although water temperature was important for initiation of migration. A number of statistically significant correlations were found between smolt ground speed and water depth, wind speed, wave height, cloud cover, barometric pressure, sun or moon visibility, and precipitation, but these were due to smolts being transported through areas with differing current velocities when changes in other environmental factors happened to occur.

No change in movement pattern was evident when smolts released in fresh water at head of tide encountered saline water upon traveling seaward, nor was a difference in behavior noted between smolts initially released at head of tide and smolts released farther downriver in saline water. Salinities ranged from 0 to 29‰ during tracking, depending upon location, tide stage, and river discharge, but smolts rarely encountered salinities >20‰ even at the lower end of the study area.

Discussion

During both years of the present study onset of increased movement of Atlantic salmon smolts released into the Penobscot River estuary corresponded to the period when hatchery and river water temperatures rose above 5°C. Earlier increased movement of smolts in 1976 coincided with an earlier rise in water temperature. Full expression of migratory behavior (i.e. regular seaward progress, no extended periods of stopping, travel in the main channel in the direction of the current during peak current flow) occurred at temperatures above 9°C. Rising water temperature has often been cited as the key environmental stimulus triggering seaward migration of Atlantic salmon smolts from freshwater streams and rivers, with peak migration occurring at temperatures of 10°C or higher (White 1939; Elson 1962; Jessop 1975; Solomon 1975). Similarly

seaward migration of steelhead trout, *S. gairdneri*, smolts (Wagner 1974) and Pacific salmon, *Oncorhynchus* sp., smolts (Foerster 1937, 1968; Hoar 1965; Hartman et al. 1967) also occurs during the period of rising water temperature in the spring. Sockeye salmon, *O. nerka*, appear to require a threshold temperature of about 5°C before seaward movement out of freshwater lakes begins (Foerster 1937, 1968), and most migrants leave by the time temperatures reach about 10°C (Foerster 1968).

The present study demonstrated that Atlantic salmon smolts travel relatively quickly through the estuary, as had been previously postulated from netting experiments (Calderwood 1908), limited direct observations (White and Huntsman 1938; Huntsman 1939; Huntsman and Hoar 1939), and tagging studies (Bartel 1976). Results from Calderwood's (1908) study implied that Atlantic salmon smolts directed their movements seaward, since Atlantic salmon smolts were captured only on ebb tide, while brown trout, *S. trutta*, smolts were caught on ebb and flood tides.

Selective tidal transport, a form of modulated drift (Harden Jones 1968; Arnold 1974) in which fishes effect transport in one direction by changing their vertical position in the water column according to tide stage, has been demonstrated in the open sea by tracking studies of the plaice *Pleuronectes platessa* (Greer Walker et al. 1978) and Atlantic cod, *Gadus morhua* (Arnold, Greer Walker, and Harden Jones, personal communication). During the present study no differences in swimming depth were noted in relation to tide stage for any smolt tracked for which depth information was obtained. Atlantic salmon smolts tracked during the present study traveled in the direction of water current flow during ebb and flood tides, drifting in the surface waters of the main channel during peak current flow. Smolts milled about, stopped moving, or apparently, moved counter to current flow when they were in shallow water or nearshore. These results seem to substantiate Huntsman's (1939, 1962, 1973) contention that smolt migration through the estuary is largely a passive, undirected event dependent upon transport with tidal current. In fact, positive rheotactic behavior occurring when smolts are in shallow water may actually delay seaward travel (Huntsman 1962).

Smolts seemed to progress seaward entirely by passive drift in the riverine position of the estuary. LaBar et al. (1978) were unable to resolve whether these smolts added active swimming to tidal transport in the open water of Penobscot Bay. Active down-estuary swimming against flooding tides or with ebbing tides could occur if smolts swam close to the bottom where tactile or visual cues might be used or close to the surface where celestial cues could be used. However, Atlantic salmon smolts usually were not making seaward progress when they were on or near the bottom, and although they did travel near the surface, turbid water and surface waves would make celestial orientation difficult or impossible. Some workers have suggested that fishes could orient to ocean currents using information obtained from geoelectric (Deelder 1952; Royce et al. 1968) or geomagnetic fields (Thornton 1932; Murray 1962). Atlantic salmon parr are able to sense weak electric fields of the magnitude that occur naturally in ocean currents (Rommel and McCleave 1973), but it has yet to be demonstrated that either geoelectric or geomagnetic cues are used in migration. Rheotaxis has never been demonstrated in fishes deprived of visual and tactile cues (Arnold 1974).

An obvious lack of behavioral changes was noted as smolts were tracked from fresh water into increasingly more saline conditions during the present study. None of the smolts tracked encountered salinities higher than 29‰ (generally <20‰) if they remained within surface waters. Atlantic salmon smolts 9–15 cm long, smaller than the size used for tracking (mean 24 cm) and therefore less resistant to comparable salinities (Huntsman and Hoar 1939; Parry 1960), can survive indefinitely when transferred directly from fresh water into salinities as high as 28‰ (Parry 1960). Complete physiological adjustment to salinities of 25–30‰, as indicated from studies on coho salmon, *O. kisutch*, smolts (Conte et al. 1966; Miles and Smith 1968), may require 36–40 h. These times agree quite well with travel times of Atlantic salmon smolts tracked from head of tide to Odom Ledge. Passive drift and intermittent milling about may have occurred while smolts became completely adapted to saltwater osmotic and ionic conditions; active swimming in Penobscot Bay may have occurred once complete physiological adjustment was attained. Other studies have shown that adult Atlantic salmon (Stasko 1975), sockeye salmon (Groot et al. 1975), and American shad, *Alosa sapidissima* (Dodson et al. 1972) migrating into freshwater rivers to spawn primarily drifted with the current when entering estuaries from the sea. In all these studies the investigators suggested that passive drift was a behavioral response related to physiological adaptation of anadromous fishes making the transition from salt to fresh water.

Shifts from passive drift to active swimming may largely be due to changing behavioral responses to different physical characteristics of the environment. Field and laboratory observations of Atlantic salmon smolts indicate that even during the freshwater phase of seaward migration, passive downstream movement (Jones 1959; Stuart 1962) as well as active downstream movement (White and Huntsman 1938; Kalleberg 1958; Stasko et al. 1973) and holding position against the current (White and Huntsman 1938; Stasko et al. 1973) may occur.

Acknowledgments

We gratefully acknowledge the cooperation of the staff of the Craig Brook National Fish Hatchery, U.S. Fish and Wildlife Service, and the Atlantic Sea-Run Salmon Commission. We thank the members of our tracking crews who served at all hours of the day and night, often under uncom-

fortable conditions: E. Albert, D. Creamer, G. Kuzmescus, J. Nichols, G. Poisson, E. Spear, T. Staples, J. Trial, and K. Turgeon. The stimulating discussions and useful suggestions of F. R. Harden Jones contributed greatly to this research. Critical reviews of the manuscript by R. J. Gibson and S. A. Rommel, Jr. are appreciated. Computer time was furnished by the Computing and Data Processing Services, University of Maine. Funds were provided by the U.S. Fish and Wildlife Service (contract no. 14-16-0008-893) and by Central Maine Power Company.

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Dechlorination of Water for Fish Culture: Comparison of the Activated Carbon, Sulfite Reduction, and Photochemical Methods[1]

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SEEGERT, G. L., AND A. S. BROOKS. 1978. Dechlorination of water for fish culture: comparison of the activated carbon, sulfite reduction, and photochemical methods. J. Fish. Res. Board Can. 35: 88–92.

Activated carbon, ultraviolet radiation, and sodium sulfite were evaluated as dechlorinating agents. Activated carbon tested in a variety of commercial dechlorinators was not 100% effective in chlorine removal. The residual concentration remaining after carbon dechlorination varied from 5 to 70 μg/L depending on how long the carbon had been in service and on the flow rate through the carbon bed. Ultraviolet radiation effectively removed residual chlorine from water but compared with the other two methods was considerably more expensive. Sodium sulfite was 100% effective but required a fail-safe injection system. A system combining the reliability of carbon dechlorination with the effectiveness of sulfite reduction was developed and shown to be safe, effective, and reliable.

Key words: water quality, toxic levels, system design, operating costs, new methods

SEEGERT, G. L., AND A. S. BROOKS. 1978. Dechlorination of water for fish culture: comparison of the activated carbon, sulfite reduction, and photochemical methods. J. Fish. Res. Board Can. 35: 88–92.

Les auteurs ont mesuré l'efficacité des procédés de déchloration au charbon activé, par radiations ultraviolettes et au sulfite de sodium. Le charbon activé, utilisé dans divers appareils de déchloration, n'a pu éliminer complètement le chlore. La concentration de chlore résiduel (entre 5 et 70 μg/L) a varié en fonction de la durée d'utilisation (état) du charbon et de la vitesse de passage sur le lit de charbon. Les radiations ultraviolettes ont éliminé le chlore résiduel, mais cette méthode s'est révélée beaucoup plus onéreuse que les autres. Le procédé au sulfite de sodium a été efficace à 100%; il nécessite toutefois un système antidéfaillance d'injection. Une méthode alliant la fiabilité de la déchloration au charbon activé et l'efficacité du sulfite de sodium a été mise au point. Elle s'est montrée sûre, efficace et fiable.

Received May 5, 1977
Accepted October 4, 1977

Reçu le 5 mai 1977
Accepté le 4 octobre 1977

MANY laboratories rely on municipal water supplies for water to hold and culture aquatic organisms. Since this water is usually chlorinated to provide proper disinfection, some form of dechlorination is necessary to render it nontoxic to aquatic organisms. Recommended allowable residual chlorine concentrations in water for holding and culturing aquatic organisms are extremely low. Brungs (1976) and U.S. Environmental Protection Agency (1975) have suggested a limit of <3 μg/L, while APHA et al. (1975) recommends an even lower limit of 0.5 μg/L. These levels are below the detection limits of the residual chlorine tests used in most laboratories. They are also well below the detection limits of commercially available chlorine monitoring systems. This means that to meet these standards the dechlorination methods chosen must be 100% effective as opposed to effective only to "below detectable limits."

Three methods of dechlorination are commonly used: activated carbon, chemical reduction, and photochemical degradation. Of these, activated carbon is the most commonly used, especially where large volumes of water must be dechlorinated. Numerous authors (Snoeyink et al. 1974; Atkins et al. 1973) have claimed that carbon is 100% effective in the removal of chlorine, while others have claimed that ultraviolet light and chemical reduction are adequate (Armstrong and Scott 1974; Beeton et al. 1976). Our objectives in this study were (1) to determine whether any of these dechlorination methods were truly 100% effective in chlorine removal and (2) to apply these results in designing a dechlorination system that could supply a large volume of chlorine-free water to maintain aquatic organisms in the laboratory.

[1]Contribution No. 123, Center for Great Lakes Studies, University of Wisconsin-Milwaukee, Milwaukee, Wis. 53201, USA.

Printed in Canada (J4813)
Imprimé au Canada (J4813)

Materials and Methods

Chlorine residuals were determined by amperometric titration (APHA et al. 1975) with modifications that permitted measurement of chlorine residuals as low as 1 μg/L (Seegert et al. 1977). The DPD method (APHA et al. 1975) was used to discriminate between free chlorine, monochloramine, and dichloramine. The lower detection limit of this method was 20 μg/L.

Experiments consisted of measuring the residual chlorine concentration in Milwaukee municipal water at the inlet and outlet of each dechlorinator over a variety of flow rates. Milwaukee municipal water contains 0.3–0.6 mg/L of residual chlorine, of which over 90% is monochloramine.

Experiments used dechlorinators with the following carbon capacities, bed depths, and maximum flow rates: Bruner-Calgon, Model AC 16FF (0.03 m^3 of 14 × 40 mesh Filtrasorb® carbon; 20 cm deep; 30 L/min); Illinois Water Treatment (I.W.T.), Models CC-230 and CM-230 (0.35 m^3 of 14 × 40 mesh Supercarb® carbon; 90 cm deep; 60 L/min); Continental H_2O Services, Model 370 (0.02 m^3 of 20 × 50 mesh "activated" carbon; 71 cm deep; 23 L/min).

A Model A600 Sanitron® UV water purifier made by the Atlantic Ultraviolet Corporation was used to determine the effectiveness of UV radiation on residual chlorine. The unit had a volume of 13.7 L and used a single 91-cm lamp rated at 39 W (14.6 W UV output) at 2537 Å.

Results

Activated Carbon

A series of tests was done using two Bruner-Calgon dechlorinators. Unit 1 contained new carbon, whereas Unit 2 had been in continuous operation for about 3 mo at a flow rate of about 12 L/min. Table 1 shows that the amount of chlorine removed is a function of how long the carbon has been in use and rate of flow through the bed. At a flow rate of 7 L/min the amount of residual chlorine remaining after dechlorination increased from 0 to 23 μg/L in 23 days. Table 1 also indicates that activated carbon accomplished 100% dechlorination for only 1 day. Further, it can be seen that at the same flow rate the residuals remaining in the 105-day-old carbon are about twice as high as in the 23-day-old carbon (e.g., 37 μg/L Unit 2 vs. 19 μg/L Unit 1; Table 1).

Coupling the two dechlorinators in a series and thereby doubling volume of carbon had little effect on the amount of chlorine removed. At a flow rate of 4.5 L/min, Unit 1 alone removed all but 10 μg/L, whereas tests the same day showed that Units 1 and 2 in series were only slightly more effective (9 μg/L remaining; Table 1). Tests the same day at higher flow rates produced similar results, indicating that the two units in series were only slightly more effective than one alone. However, when the two units were in series, increases in flow rate from 4.5 to 17 L/min had little effect on the residual concentrations remaining, reflecting the influence of the increased carbon volume (Table 1).

The detailed observations described above are supported by some general observations using two Continental H_2O Services dechlorinators connected in series. Chlorine measurements taken 1–2 days after the initial start-up of these dechlorinators indicated that at a flow rate of 4–7 L/min a residual of 10–15 μg/L of chlorine was passing through. Measurements taken about 8 mo later indicated that at a flow rate of 2 L/min about 20–30 μg/L of residual chlorine was being passed through.

Observations on a large capacity dechlorinator (I.W.T. Model CC-230) confirm the pattern seen in the smaller dechlorinators. Initial measurements taken <1 h after start-up indicated that at a flow rate of 60 L/min, 5 μg/L of chlorine passed through the dechlorinator. However, the amounts passed through quickly increased to 10 and 15 μg/L after 2 and 24 h, respectively. Measurements taken after 6 mo of continuous operation indicated that at a flow rate of about 38 L/min, 40–50 μg/L of chlorine was passed through the dechlorinator. Observations on a similar-sized I.W.T. dechlorinator (Model CM-230) indicated that after about 3 yr of continuous operation 70 μg/L of chlorine was passing through the dechlorinator when

Table 1. Carbon dechlorination tests at various flow rates. ND = not determined.

Unit tested	Days in service	Initial chlorine concn (μg/L)	Flow rate (L/min) 4.5	7	11	15	17	20
			Residual chlorine concn (μg/L)					
1	0	ND		0		1–2		
1	2	ND		8				
1	3	ND		10				
1	8	128	10		12			16
1	23	157	19	23				
2	~90	128	25		33			34
2	~105	157	37					
1 & 2[a]	0	128	9		11		11	

[a]Units 1 and 2 in series.

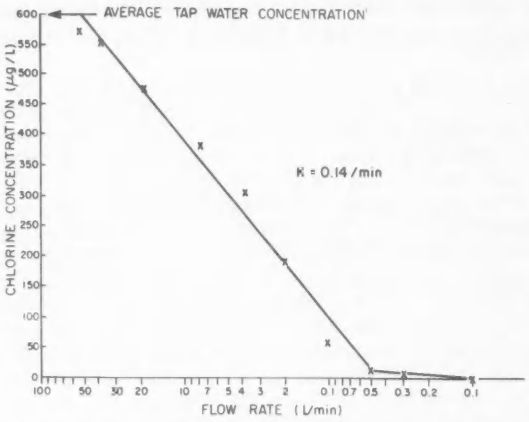

FIG. 1. The reduction of residual chlorine in tap water at different flow rates through an ultraviolet dechlorinator.

the inflow water contained 590 μg/L.

We initially thought that the water flow through the carbon bed might be creating channelization, reducing the carbon surface area available for dechlorination. However, backwashing had no noticeable effect on the efficiency of chlorine removal. Thus, the reduced efficiency of chlorine removal results from some change in the carbon itself and not merely from changes in flow characteristics through the carbon bed.

ULTRAVIOLET TESTS

Tests using the A600 UV sterilizer indicated that UV radiation can reduce residual chlorine concentrations to zero. We found that initial chlorine concentrations of about 0.6 mg/L were reduced by 50, 90, and 99% at flow rates of 4, 0.9, and 0.2 L/min, respectively (Fig. 1). At flow rates of 0.5–50 L/min the reduction of monochloramine followed first-order kinetics (Fig. 1). The rate constant (K) determined according to the equation shown below was 0.14/min.

$$K = \frac{\ln C_0 - \ln C}{t}$$

where C_0 = initial chlorine concentration; C = chlorine concentration at time t; t = time in UV chamber.

Discussion

The most important finding of this study was that except for a very short initial period activated carbon is not 100% effective in removal of chlorine from water. New activated carbon placed in dechlorinators was only 100% effective for a few hours. Within a few days 10–20 μg/L of residual chlorine was typically being passed through the dechlorinator.

These results at first appear to be contrary to the findings of others (Atkins et al. 1973; Bauer and Snoeyink 1973; Snoeyink et al. 1974), who have indicated that activated carbon can completely remove residual chlorine. The apparent disparity is probably related to what constitutes a "measurable residual" (i.e. what are the detectable limits). Most of the studies on the effectiveness of activated carbon as a dechlorinating agent have been related to its use in wastewater treatment where residual chlorine concentrations are typically in the range of 1–5 mg/L (Snoeyink and Markus 1973). Measurement techniques appropriate to this concentration range are employed with the lower detection limit frequently being 0.1 mg/L. Values below this are routinely recorded as zero. Although these detection levels may be adequate for problems related to wastewater treatment they are not adequate to accurately measure chlorine residuals in the micrograms per litre range. Thus statements in the literature such as "the effluent [was] essentially void of residual chlorine" (Bauer and Snoeyink 1973), "an immeasurable amount of chlorine remained" (Snoeyink et al. 1974), or "dechlorination by this method [activated carbon] is for all practical purposes complete" (White 1972) frequently appear in the wastewater treatment literature and should be viewed with caution.

Two other factors may be contributing to the discrepancy in findings. First, many reports (e.g. Hager and Flentje 1965; Suidan 1975) are based on the ability of activated carbon to remove free chlorine as opposed to monochloramine. Snoeyink and Markus (1973) have shown that the reaction rate for the removal of monochloramine is about 10 times slower than for free chlorine. This is especially important in the context of this paper, since most municipal water contains a large percentage of monochloramine. The second factor may be that some investigators have based their results solely on short-term static batch tests which lead to the erroneous assumption that chlorine removal is always complete. Errors may be compounded by extrapolating short-term results into long-term predictions involving the effective life of the carbon.

ULTRAVIOLET EFFECTS

Free chlorine when exposed to UV radiation is rapidly reduced in accordance with the following equation: $HOCl = H^+ + Cl^- + \frac{1}{2}O_2$ (Hancil and Smith 1971). Chloramines and chlororganics, though less reactive, have also been reported as susceptible to reduction when irradiated by UV light (Snoeyink and Markus 1973; Armstrong and Scott 1974; Southworth and Gehrs 1976). Since the chlorine residual in Milwaukee municipal water is entirely as chloramines, our studies support the findings of Armstrong and Scott (1974), who demonstrated that UV radiation could be used for dechlorination of municipal water. Although we have demonstrated that 100% chlorine removal is possible, several drawbacks to a UV system should be

pointed out. Since most municipalities chlorinate at about 500 μg/L at least 99% chlorine removal is necessary for proper dechlorination. In our studies 99% reduction occurred at a flow rate of only 0.2 L/min indicating that a much larger UV system would be necessary. Armstrong and Scott (1974) found that at a flow rate of 50 L/min two 1200-W sterilizers removed 90% of the free chlorine from municipal water. Two 1200-W sterilizers (Xodar XO-260) presently cost $14,000. The manufacturer recommends lamp replacement every 8000 h (~1 yr), which would cost about $1,000·unit^{-1}·yr^{-1}. Electricity costs of $850·yr^{-1} ($0.04 kW-h) would raise the annual operating costs to $2,850.

Armstrong and Scott (1974) reported that dechlorination continued after irradiation but could not explain this phenomenon. We offer a possible explanation. Many UV lamps have the capacity to generate hydrogen peroxide (H_2O_2). Hydrogen peroxide is itself a dechlorinating agent which reacts with chlorine according to the following equation: $HOCl + H_2O_2 = O_2 + H^+ + Cl^- + H_2O$ (Mischenko et al. 1960). Thus the delayed dechlorination reported by Armstrong and Scott is probably a result of hydrogen peroxide slowly reacting with hypochlorous acid.

Dechlorination by Chemical Reduction

Chemical reducing agents, principally sulfur compounds (e.g. $Na_2S_2O_3$, Na_2SO_3, SO_2), have long been used for dechlorination (Rodgers 1949; Pyle 1960). They are effective and relatively nontoxic to aquatic organisms (Pyle 1960; Beeton et al. 1976). Beeton et al. (1976) screened a variety of reducing agents and concluded that sodium sulfite (Na_2SO_3) was safest and most economical for dechlorination. They also found that sodium sulfite eliminated the toxicity of residual chlorine to aquatic invertebrates. We have found that sodium sulfite used at a 2:1 ratio (by weight) with residual chlorine completely and instantaneously reduced chloramines. We also found that the dechlorinated water was nontoxic to a wide variety of fish.

Dechlorination by the use of reducing agents, although completely effective, has one drawback. A highly accurate and fail-safe injection system must be devised. Periodic corrections may also be necessary to compensate for seasonal or even daily chlorine fluctuations in municipal water.

Carbon–Sulfite System

Since each method used alone has inherent drawbacks (carbon effectiveness; UV cost; sulfite-injection system) we developed a combination method which is effective, reliable, and relatively inexpensive. Municipal water, up to 60 L/min, is first routed through a I.W.T. Model CC-230 dechlorinator. This provides 90–95% chlorine removal. A sodium sulfite solution is then injected (via a Cole Parmer Master Flex® Pump Model #8545) into the water discharged from the dechlorinator. The sulfite solution (5 g/L) is pumped from a 19-L carboy directly into the line carrying the carbon-dechlorinated water to our culture tanks. To compensate for decomposition of the sodium sulfite and provide an adequate margin of safety we have found that the ratio of Na_2SO_3 to residual chlorine should be about 6:1 (by weight). We assume that .05 mg/L of residual chlorine will pass through the carbon dechlorinator, so the sodium sulfite is added at a rate of 0.3 mg Na_2SO_3/L of carbon-dechlorinated water. At the maximum flow rate (60 L/min) about 26 g of sulfite is used per day. Although we are adding excess quantities we have not detected sulfite in our fish holding tanks or noticed any decrease in oxygen levels.

An important feature of this system is its nearly fail-safe nature. In the event of an undetected pump failure water continues to flow through the carbon bed which by itself can effect 90% dechlorination. This will prevent any loss of fish until the pump can be repaired or replaced.

Using current prices we have calculated the approximate cost of this system. Based on past experience we are projecting a carbon-bed life of about 2 yr.

Equipment		Operating costs — 1 yr	
IWT CC-230 dechlorinator (including carbon)	$2065.00	Carbon .35 m³ × $860/m³ = $300/2	= $150
Masterflex® 8545 pump (including head)	225.00	Na_2SO_3 21 lb/yr × $1/lb =	21
	$2290.00		$171

For laboratories that already have a carbon dechlorination system, installing a sulfite pump would require minimal expense.

Our carbon–sulfite dechlorination system has thus far provided one year of trouble-free and effective dechlorination.

Acknowledgments

This research was supported by a grant from the American Electric Power Service Corporation, Consumers Power Company, Northern Indiana Public Service Company, Wisconsin Electric Power Company, Wisconsin Public Service Corporation, and Commonwealth Edison Company.

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Seawater Adaptation and Parr–Smolt Transformation of Juvenile Atlantic Salmon, *Salmo salar*

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FARMER, G. J., J. A. RITTER, AND D. ASHFIELD. 1978. Seawater adaptation and parr–smolt transformation of juvenile Atlantic salmon, *Salmo salar*. J. Fish. Res. Board Can. 35: 93–100.

Seawater adaptation of 1- and 2-yr-old Atlantic salmon, *Salmo salar*, presmolts and smolts was assessed during exposure to salinity increasing from 0.1 to 31‰ by examining changes in the osmotic concentration of their serum, urine, and intestinal fluid. Both age-classes adapted to seawater equally well; the marine osmoregulatory mechanisms apparently function before completion of parr–smolt transformation. Adaptation was possible during the parr stage for fish exceeding 12–13 cm fork length. The timing of transformation was synchronous for 1- and 2-yr-old juveniles as demonstrated by changes in their lipid and moisture content and condition factor (K). Condition factor of juveniles at the Mactaquac Hatchery, New Brunswick (45°58′N), decreased from February until the second half of May, while lipid content decreased from February until the second half of June. Migratory activity of juveniles at this location was maximal during the second half of May coincident with attainment of minimum K values and decreased during the first half of June as K values increased. While timing of changes in lipid and moisture content and K values were similar for juveniles at the Mersey Hatchery, Nova Scotia (44°10′N), lipid content of fish at the more southerly station decreased at a greater rate and migratory activity was apparent earlier. This was probably attributable to the advanced temperature cycle at that location. Smolt-release dates are discussed in terms of the development of the marine osmoregulatory mechanisms and the timing of parr–smolt transformation.

Key words: Atlantic salmon, seawater adaptation, parr–smolt transformation, migration, hatchery management

FARMER, G. J., J. A. RITTER, AND D. ASHFIELD. 1978. Seawater adaptation and parr–smolt transformation of juvenile Atlantic salmon, *Salmo salar*. J. Fish. Res. Board Can. 35: 93–100.

Les auteurs ont étudié l'adaptation à l'eau de mer des saumons atlantique (*Salmo salar*) de 1 et de 2 ans (stade saumoneau et antérieur) soumis à des augmentations de 0,1 à 31‰ de la salinité, en mesurant les variations de la concentration osmotique de leur plasma, de leur urine et de leur liquide intestinal. Les deux classes d'âge se sont également bien adaptées; les mécanismes de la régulation osmotique en milieu marin fonctionnent apparemment avant la fin de la smoltification. Les tacons qui mesuraient plus de 12 à 13 cm, du nez à la fourche ont réussi à s'adapter. La transformation a été synchrone chez les jeunes de 1 et de 2 ans comme le démontrent les variations de la teneur en lipides et en eau et du facteur K. Dans le cas des jeunes saumons de la piscifacture de Mactaquac, au Nouveau-Brunswick (45°58′N), le facteur K a diminué à partir de février jusqu'à la deuxième moitié de mai, tandis que la teneur en lipides a baissé à partir de février jusqu'à la deuxième moitié de juin. L'activité migratoire des jeunes saumons de cet endroit a été la plus intense, durant la deuxième moitié de mai, au moment où les valeurs de K étaient les plus faibles, et elle a diminué au cours de la première moitié de juin tandis que les valeurs de K augmentaient. Alors que le synchronisme des variations de la teneur en eau et en lipides et des valeurs de K ont été similaires chez les jeunes saumons de la piscifacture de Mersey, en Nouvelle-Écosse (44°10′N), la teneur en lipides y a baissé beaucoup plus rapidement et l'activité migratoire y a débuté plus tôt. La précocité du cycle de la température à cet endroit en est probablement la cause. Les auteurs examinent les dates du renvoi des saumoneaux à la mer, compte tenu de la mise en place des mécanismes osmorégulateurs et du moment de la smoltification.

Received July 5, 1977
Accepted October 6, 1977

Reçu le 5 juillet 1977
Accepté le 6 octobre 1977

Printed in Canada (J4910)
Imprimé au Canada (J4910)

JUVENILE Atlantic salmon, *Salmo salar*, for release in the Maritimes are reared at hatcheries in diverse geographic locations which have different water temperature regimes. The differences in temperature result in variations in salmon growth rate. Juveniles, reared at the Charlo Hatchery in northern New Brunswick (47°58′ N), are held for 2 yr before they are large enough to undergo parr–smolt transformation and be liberated during the spring. In contrast, a proportion of the juveniles at the Mactaquac Hatchery, N.B. (45°58′ N), undergo transformation as yearlings. The remainder of the juveniles at this location are retained for an additional year and liberated as larger 2-yr-old smolts. Because of the warmer temperature regime encountered at the Mersey Hatchery in southwest Nova Scotia (44°10′ N), virtually the entire production at this location during 1976 and 1977 has been of yearling smolts.

Adult return rate resulting from the release of 2-yr-old smolts in the LaHave River, N.S., during 1972-74 was threefold greater than the rate resulting from the liberation of yearling smolts during those years. This indicated a need for information of the timing of transformation of both juvenile age-classes and of the time and size at which their marine osmoregulatory mechanisms develop to aid in determining hatchery-release dates. The experiment was conducted using juveniles at Mactaquac and Mersey because these hatcheries are the largest producers of smolts in the Maritimes; juveniles from Mactaquac are released in the Saint John River (N.B.) and those from Mersey in the LaHave River (N.S.). Additionally, the difference in temperature cycle between these locations was expected to demonstrate the influence of this variable on the timing of migratory activity and transformation.

Materials and Methods

SEAWATER ADAPTATION OF PRESMOLTS AND SMOLTS

Changes in the osmotic concentration of serum, urine, and intestinal fluid of euryhaline teleosts during exposure to increasing salinity have been documented (Parry 1966; Conte 1969; Hickman and Trump 1969; Shehadeh and Gordon 1969) and were used as criteria for determining the success of seawater adaptation.

The 1- and 2-yr-old juvenile salmon ($\geq$14 cm) used in the seawater adaptation study were exposed to increasing salinity during February–March as presmolts and again during May as smolts. Salmon of both age-classes were progeny of spring-run parents captured in the Saint John River and were obtained from the Mactaquac Hatchery during early February and again during late April. Upon arrival at the laboratory, 100 juveniles of both age-classes were added to each of four 3000-L circular freshwater tanks (4±0.5°C); the two age-classes within a tank were separated by a partition made of small mesh netting. The salmon were exposed to a natural photoperiod and fed to satiation daily with finely chopped Atlantic cod, *Gadus morhua*. The mean weights and fork lengths (with 1 SD in parentheses) of the February and May groups were

	February		May	
Age (yr)	Fork length (cm)	Wt. (g)	Fork length (cm)	Wt. (g)
1	15.4 (0.6)	36.4 (4.4)	16.6 (0.9)	41.0 (6.7)
2	21.9 (1.1)	105.8 (15.4)	21.0 (1.0)	102.2 (18.9)

During the February and May studies, four 1- and four 2-yr-old juveniles, deprived of food for 72 h, were randomly selected from each of the three freshwater tanks to obtain samples of their blood, urine, and intestinal fluid. The salinity within each tank was increased 2‰·day^{-1}, and 12 salmon of both age-classes were sampled at 15.5 and 31‰. The salmon were then held for an additional month at 31‰ and a record was made of any mortalities. A gradual rather than an abrupt exposure to seawater (31‰) was chosen because migrating smolts presumably have the ability to control salinity change. Fish in the fourth tank served as controls and were held in fresh water until September at temperatures similar to those at Mactaquac. Twelve juveniles of each age-class were removed monthly from the control tank and killed in order to follow changes in condition factor ($K = W/l^3$) and proximate composition. Weight of these fish (W) was recorded in grams and fork length (l) in centimetres.

Upon removal from the tanks, salmon were placed on their dorsal surface and the cloacal area rinsed with deionized water and blotted dry. Urine was collected by placing the tapered tip of a microcapillary tube against the urinogenital papilla and applying gentle pressure on the bladder (Shehadeh and Gordon 1969). The cloaca was again rinsed with deionized water and blotted dry before collecting intestinal fluid by inserting a microcapillary tube into the hindgut. Blood samples, taken from the caudal artery by syringe, were transferred to microcapillary tubes which were sealed at one end and left for ½ h at room temperature. Capillaries were then centrifuged in a microhematocrit centrifuge for 5 min and the portion containing the serum separated and sealed. Osmolarity of urine and intestinal fluid was determined immediately after collection and that of serum within 3 h with a Clifton nanolitre osmometer. Salmon were not anesthetized before sampling their body fluids.

SEAWATER ADAPTATION OF PARR

The osmoregulatory ability of salmon parr (2–37 g) was assessed during June using 200 underyearling fish from Mactaquac. The parr were held in a 3000-L freshwater tank at 15±0.5°C and fed chopped cod once daily. Measurements of total body moisture and osmolarity of serum and intestinal fluid were made for 15 individuals randomly selected from the tank. Salinity was then increased 2‰·day^{-1} and 15 parr sampled at 15.5‰ and after 5 days exposure to 31‰. The remaining parr were held for 2 mo at that salinity and a record kept of the size of the individuals that died. This procedure was repeated during November with a different group of parr.

TIMING OF PARR–SMOLT TRANSFORMATION

The timing of migratory activity and changes in condition factor and proximate composition associated with parr–smolt transformation (Wagner 1968; Fessler and Wagner

1969) were determined for salmon at the Mactaquac and Mersey hatcheries during the winter and spring. Migratory activity was assessed from the tendency for smolts to swim with rather than against the current in the rearing ponds as observed for pre- and postsmolts. Thirty juveniles were randomly removed from each of four ponds at Mactaquac on seven sampling dates between February and June for weight and fork length measurements and 15 fish from each pond were killed on all dates for lipid and moisture determinations. Two of the ponds contained 2-yr-old juveniles and the other two, yearlings; all fish were progeny of spring-run parents captured in the Saint John River. Similarly, weights and lengths of 40 juveniles from each of two ponds at Mersey were measured on five dates between February and May and 15 individuals from the ponds were killed on each date for lipid and moisture determinations. All of these juveniles were yearlings and were progeny of adults captured in the LaHave River. Continuous recording of water temperature at both hatcheries was made during the study for comparison with data from previous years.

Proximate Analysis

Moisture content was determined by freeze-drying whole fish to constant weight. The dried fish were then ground in a laboratory mill and lipid determined from 1-g subsamples by extraction for 4 h with chloroform–methanol (2:1) (Folch et al. 1957). Ash content was measured by heating 1-g subsamples at 550°C for 24 h (AOAC 1970). The ammonia released after Kjeldahl digestion was measured with an ammonia-sensing gas electrode (Orion #95-10) (S. Ray, Dep. Fish. Environ., Biological Station, St. Andrews, N.B., personal communication) to provide a measure of nitrogen content. Protein content was calculated by multiplying the nitrogen estimates by 6.25.

Data Analysis

Data were subjected to stepwise multiple regression analysis to determine the relationship between the dependent and independent variables; the acceptance of a given independent variable was judged by its contribution to the minimization of the residual mean square (Draper and Smith 1966).

Results and Discussion

Seawater Adaptation

During the February and May studies, serum osmolarity of the 1- and 2-yr-old juveniles ($\geq$14 cm) showed a gradual linear increase of about 7% as salinity increased from fresh water (0.1‰) to 31‰. The relationship between serum osmolarity and ambient salinity was described by the regression

$$(1)\quad O_s = 0.769S + 333.283 \quad (R^2 = 0.405;\ \mathrm{df} = 1, 191),$$

where O_s is serum osmolarity (milliosmoles per litre) and S is salinity (parts per thousand). Neither month of seawater exposure nor age of the juvenile salmon significantly influenced changes in osmolarity of their serum during exposure to increasing salinity ($P > 0.05$).

Intestinal-fluid osmolarity of both juvenile age-classes also showed a small linear increase (10%) as salinity increased from 0.1 to 31‰. The following regression of ambient salinity (S) on osmolarity of intestinal fluid (O_I) was used to describe the relationship

$$(2)\quad O_I = 1.009S + 305.649 \quad (R^2 = 0.263;\ \mathrm{df} = 1, 144).$$

Neither month of exposure to increasing salinity nor salmon age significantly influenced osmolarity of this fluid during salinity change.

Increases in salinity from 0.1 to 31‰ resulted in an exponential increase in the osmolarity of urine collected from both age-classes of juveniles. Osmolarity of this fluid from individuals in 0.1‰ was similar to that of their medium, whereas values for salmon in 31‰ were similar to those observed for serum and intestinal fluid. Urine osmolarity for both age-classes was slightly greater at 0.1 and 15.5‰ during May than during February, and at 31‰ it was identical on both occasions. The following regression of salinity (S) and month of the year (M) on urine osmolarity (O_u) was used to describe the data:

$$(3)\quad \log_{10} O_u = 0.036S + 0.249M - 0.008(S \times M) + 1.424 \quad (R^2 = 0.943;\ \mathrm{df} = 3, 165).$$

The month term was a dummy variable, where 0 represented the February study and 1 the May study. Juvenile age was not significant in the analysis.

During the February and May studies 2-yr-old juveniles exhibited 100% survival in all tanks, while 0–8% of the yearlings died. Mortality among yearlings was attributable to scale loss and wounding resulting from aggressive behavior. Osmotic concentrations of serum and intestinal fluid obtained from parr inadvertently included in the seawater tanks were elevated. Values of 495(41) and 471(49) mOsm·L^{-1} (with 1 SD in parentheses) for serum and intestinal fluid, respectively, were 50% greater than values recorded in fresh water. Changes in osmolarity of the body fluids of juveniles ($\geq$14 cm) and of salmon parr which accompanied increases in salinity are shown in Fig. 1.

Exposure of parr to 31‰ demonstrated that mortality was restricted to fish $\leq$19 g ($<$12 cm fork length) and was greatest among the smallest individuals within this size range. Parr, unable to adapt to 31‰, showed increases in osmolarity of their serum and intestinal fluid similar to those previously described coupled with decreased body moisture (5%). For parr $>$19 g ($>$12 cm), no signs of osmotic stress were apparent. This relationship between serum osmolarity (O_s), parr weight in grams (W), and ambient salinity (S) was described by the regression

$$(4)\quad O_s = 0.180S^2 - 0.011(S^2 \times W) + 0.201 \times 10^{-3}(S^2 \times W^2) + 315.998 \quad (R^2 = 0.716;\ \mathrm{df} = 3, 92).$$

Serum osmolarity of parr in fresh water remained constant over the 2–37-g weight range, changing little with increases in salinity to 15‰. At 31‰, elevation of

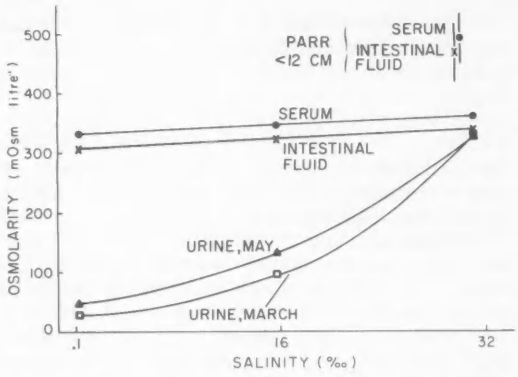

FIG. 1. Changes in the osmotic concentration of serum, urine, and intestinal fluid of juvenile Atlantic salmon ($\geq$14 cm) exposed to increasing salinity. Values were calculated from equations 1, 2, and 3. Mean values $\pm$1 SD for parr of <12 cm in 31‰ salinity given for comparison.

osmolarity above values observed for fish in fresh water was most marked among the smallest individuals (52% increase), whereas values for the heaviest parr showed little increase (6%) (Fig. 2).

Osmolarity of serum and intestinal fluid of euryhaline teleosts adapted to seawater increases by only $\leq$10% above freshwater concentrations (Parry 1961, 1966; Shehadeh and Gordon 1969). The relatively small changes in osmolarity of serum and intestinal fluid observed for juveniles ($\geq$14 cm) during February and May indicate that both age-classes adapted to seawater equally well and that completion of parr–smolt transformation was not required for adaptation. Accordingly, salinity was the only significant variable describing changes in osmolarity of these fluids, whereas salmon age and month of seawater exposure were nonsignificant. While month of seawater exposure did not appear to influence the ability of juvenile salmon to adapt to 31‰, fish used in both studies were markedly different (Table 1). Those employed during February had high lipid and K values characteristic of presmolts (Saunders and Henderson 1970), whereas juveniles used during May had lower lipid and K values distinctive of smolts.

Changes in urine osmolarity of both juvenile age-classes during exposure to increasing salinity also followed a pattern typical of a euryhaline teleost possessing the capacity for marine hypoosmoregulation (Hickman and Trump 1969). Again, the most sig-

TABLE 1. Changes in condition factor (K), weight (in grams), and proximate composition (percent wet weight) of 1- and 2-yr-old juvenile Atlantic salmon during the period 56–258 days from January 1. Each value is a mean of 12 fish with 1 SD in parentheses.

	Days from January 1							
	56 (Feb. 25)	84 (Mar. 24)	126 (May 5)	139 (May 18)	147 (May 26)	168 (June 16)	198 (July 16)	258 (Sept. 14)
				1-yr-olds				
K	.991	.981	.906	.890	.916	.889	.932	1.042
	(.049)	(.060)	(.038)	(.035)	(.045)	(.030)	(.029)	(.068)
Moisture	72.543	73.460	74.281	75.092	76.139	75.810	76.850	72.377
	(.626)	(1.201)	(.544)	(.655)	(.770)	(.619)	(.843)	(1.240)
Protein	16.608	15.695	16.385	17.181	15.647	16.573	16.853	18.439
	(.757)	(.673)	(.499)	(.629)	(.674)	(.734)	(.273)	(.867)
Lipid	8.283	8.725	7.007	6.060	5.938	5.202	4.106	7.088
	(.667)	(.933)	(.524)	(.556)	(.526)	(.599)	(.591)	(1.114)
Ash	2.478	2.257	2.468	2.357	2.312	2.326	2.300	2.401
	(.075)	(.153)	(.068)	(.072)	(.065)	(.056)	(.071)	(.067)
Weight	35.617	34.978	40.307	45.528	46.853	51.915	86.443	188.528
	(4.126)	(5.599)	(6.888)	(5.949)	(8.007)	(6.472)	(10.265)	(21.346)
				2-yr-olds				
K	1.009	.996	.956	.897	.905	.863	.889	1.034
	(.044)	(.060)	(.050)	(.041)	(.048)	(.017)	(.039)	(.174)
Moisture	71.832	72.927	74.120	73.123	75.166	74.669	76.314	72.836
	(.688)	(.649)	(1.108)	(1.057)	(1.043)	(.866)	(1.481)	(.653)
Protein	18.950	16.536	17.938	18.899	17.106	17.939	17.802	18.222
	(.830)	(.582)	(.494)	(.344)	(.743)	(.570)	(.417)	(.894)
Lipid	7.631	7.619	6.190	6.274	5.365	4.933	3.759	6.554
	(.798)	(.792)	(.969)	(1.052)	(.893)	(.678)	(1.147)	(.629)
Ash	2.498	2.460	2.497	2.496	2.413	2.490	2.459	2.366
	(.095)	(.084)	(.095)	(.119)	(.135)	(.085)	(.050)	(.032)
Weight	106.429	93.198	99.810	105.627	97.192	115.922	161.460	252.321
	(12.811)	(10.164)	(18.056)	(16.655)	(16.053)	(16.055)	(22.014)	(15.484)

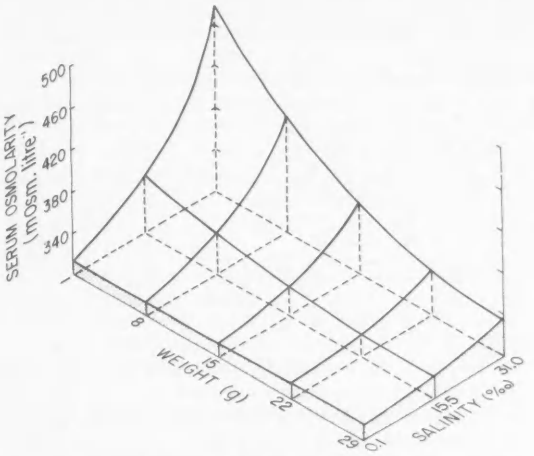

FIG. 2. The influence of ambient salinity on the serum osmolarity of underyearling Atlantic salmon parr of various size. Values calculated from equation 4.

nificant variable describing changes in osmolarity was ambient salinity, whereas month of exposure explained only a small percentage of the variation and salmon age was nonsignificant.

Seawater adaptation of Atlantic salmon was possible during the parr stage for fish in excess of 12 cm fork length, in agreement with Wagner's (1974) observation that seawater adaptation of juvenile rainbow trout, *Salmo gairdneri*, preceded completion of parr–smolt transformation and occurred when trout reached a length of 12 or 13 cm. Similarly, Knutsson and Grav (1976) observed that Atlantic salmon that had attained a length of 11–14 cm survived in seawater. Conte (1969) has suggested that transformation is associated with a type of biological clock informing smolting species of the time to seek the marine environment rather than representing the development of the marine hypoosmoregulatory mechanisms. However, Saunders (R. L. Saunders, North American Salmon Research Center, St. Andrews, N.B., personal communication) has observed that median survival time of juvenile Atlantic salmon transferred directly to 40‰ from fresh water on various dates between January and June increases as they undergo parr–smolt transformation. Thus, while salmon parr >12 cm are apparently able to regulate their body fluids in seawater, further development of the osmoregulatory mechanisms enabling them to thrive in this medium may occur during transformation (Saunders and Henderson 1970).

Holmes and Stainer (1966) observed during transformation of rainbow trout that rate of urine flow and ionic excretion in fresh water declined to half presmolt values and that these changes were accompanied by increases in urine osmolarity; this phenomenon was observed in our study for juveniles held in 0.1 and 15.5‰

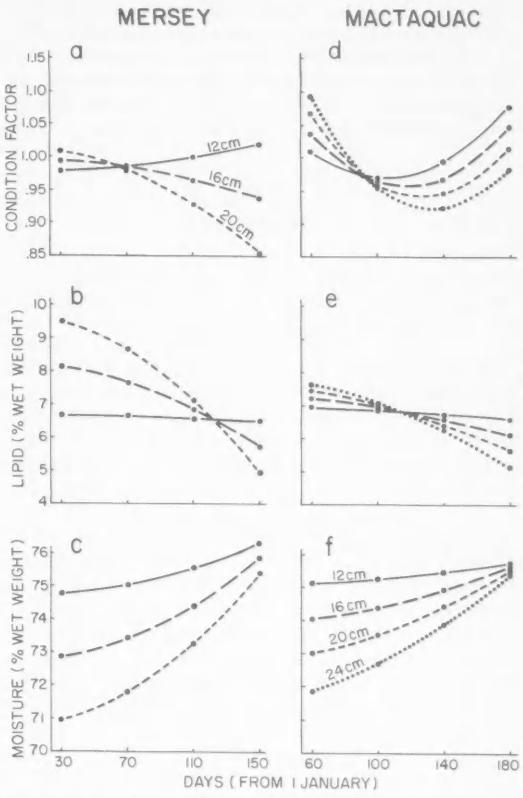

FIG. 3. a–f, The relationship between time of year (cumulative number of days from January 1) and changes in condition factor and lipid and moisture content of juvenile Atlantic salmon of various size. Values calculated from equations in Table 2.

during transformation. The changes occurring in the renal excretory pattern of trout during transformation tended towards the pattern known to exist for seawater-adapted fish and was interpreted as a preadaptation to the marine environment. Accordingly, increases in urine osmolarity during May for salmon held in fresh water and half seawater were coincident with their downstream migratory period.

TIMING OF PARR–SMOLT TRANSFORMATION

Mersey Hatchery — Condition factor of 16–20-cm juveniles declined curvilinearly from the first sampling date in January (day 30) until the end of May (day 150) (Fig. 3a) when the last groups of fish were released. The extent of the decline in K values was greatest for the larger juveniles. In contrast, condition of juveniles $\leq$12 cm showed a gradual increase throughout the experimental period.

Lipid content of 16–20-cm juveniles also showed a

TABLE 2. Regressions of the cumulative number of days from January 1 (D) and salmon fork length (FL) on changes in condition factor (K), lipid (L), and moisture content (M) of juvenile salmon at the Mactaquac and Mersey hatcheries. Lipid and moisture are expressed as percent wet weight and fork length in centimetres.

Hatchery	Regression	R^2	df
Mersey	$K = 0.152 \times 10^{-4}D^2 + 0.004\text{FL} - 0.111 \times 10^{-5}(D^2 \times \text{FL}) + 0.927$	0.213	3,410
	$L = 0.301 \times 10^{-3}D^2 + 0.375\text{FL} - 0.257 \times 10^{-4}(D^2 \times \text{FL}) + 2.195$	0.520	3,145
	$\text{M} = -0.120 \times 10^{-3}D^2 - 0.485\text{FL} + 0.162 \times 10^{-4}(D^2 \times \text{FL}) + 80.511$	0.571	3,145
Mactaquac	$K = 0.855 \times 10^{-5}D^2 + 0.024\text{FL} + 0.902 \times 10^{-6}(D^2 \times \text{FL})$ $- 0.341 \times 10^{-3}(D \times \text{FL}) + 0.897$	0.231	4,745
	$L = 0.600 \times 10^{-4}D^2 + 0.080\text{FL} - 0.612 \times 10^{-5}(D^2 \times \text{FL}) + 6.078$	0.242	3,311
	$\text{M} = -0.785 \times 10^{-4}D^2 - 0.299\text{FL} + 0.832 \times 10^{-5}(D^2 \times \text{FL}) + 78.678$	0.366	3,311

curvilinear decline between January and May but remained relatively constant for fish $\leq$12 cm (Fig. 3b). For the larger juveniles (20 cm), lipid content was about 45% lower at the end of May (day 150) than values recorded at the end of February (day 60), while lipid of those smaller (16 cm) was 27% lower (decrease as percent of initial value). More than half these changes in lipid occurred during late April and May when migratory activity was apparent in the rearing ponds. Decreases in lipid were accompanied by increases in moisture content (Fig. 3c).

Migratory activity at Mersey was first apparent by mid-April (day 107) coincident with an increase in water temperature from 6 to 8°C and continued until the end of May (day 150) when the last groups of juveniles were released. The largest fish demonstrated this activity a few days earlier than smaller fish, but size differences were not apparent later in the migratory period. Water temperatures at Mersey usually increase to 4°C at the first of April, reaching 10°C by the first of May and 15°C at the end of that month.

Mactaquac Hatchery — Condition factor of 16–24-cm salmon decreased from the first sampling date in February until late May (about day 140) when values began to increase (Fig. 3d). Within this size range, the magnitude of the decrease in K value was greatest for the larger fish. The K values for juveniles $\leq$12 cm remained relatively constant until about the beginning of May (day 120) when they began to increase. The response surface describing changes in condition does not show the gradual curvilinear decline evident between February and May. However, more importantly, it demonstrates that K starts to increase during late May.

Lipid content for juveniles of various size showed a gradual curvilinear decline during the experimental period (Fig. 3e), the magnitude of the decline being greatest for the larger salmon. For example, lipid content of 20-cm juveniles was about 16% lower at the end of May (day 150) than values recorded at the end of February (day 60), while values for 16-cm fish were 10% lower (decrease as percent of initial level). Changes in moisture content during the experimental period were the inverse of those observed for lipid (Fig. 3f).

Migratory activity or reverse orientation to the current in the holding ponds occurs during May at Mactaquac, particularly the last half of that month, and ceases by early June (J. McAskill, Dep. Fish. Environ., Mactaquac Hatchery, Fredericton, N.B., personal communication). Mean daily water temperatures at this location usually reach 5°C at the beginning of May, increasing to 10°C at the end of that month and to 15°C by mid-June.

Changes in condition factor and lipid and moisture content of juveniles at Mactaquac and Mersey were modulated by time of year (cumulative days from January 1) acting quadratically, necessitating the use of the Day^2 variable in the regression analysis (Table 2). Additionally, because these changes were size specific, fork length and the fork length $\times$ Day^2 terms were also independent variables in the analysis.

The greater part of natural mortality of juvenile salmonids occurs during the first few weeks or months after the start of downstream migration (Carlin 1968; Ricker 1976). For cultured juveniles, evidence suggests that factors such as timing of release, increased size and energy reserves, diet, physical training, and lower disease incidence are important in decreasing this initial period of high mortality (Carlin 1968; Wagner 1968; Burrows 1969; Peterson 1973; Wendt and Saunders 1973). In our study, attention was devoted to predicting hatchery-release dates by examining changes associated with parr–smolt transformation, a process largely regulated by photoperiod and temperature (Wagner 1974). Of particular importance were changes in energy levels and migratory disposition of juvenile salmon.

Despite a 50% decrease in lipid content during transformation and a concomitant increase in moisture, protein content remained relatively constant (Table 1), indicating that energy expenditure associated with transformation is largely satisfied by lipid. Lipid continued to decrease for about 30 days after K values began to increase, allowing protein deposition during a period of isometric growth. Since energy reserves decrease during transformation, smolt liberation earlier

within the migratory period ensures energy availability that may reduce mortality during adaptation to different conditions of survival and feeding in a new environment. Accordingly, Peterson (1973) reported a higher rate of returning adult Atlantic salmon from the release of smolts fed a diet of 16% lipid content than from the release of smolts offered a 6% lipid diet. Similarly, Burrows (1969) observed a greater adult return of chinook salmon, *Oncorhynchus tshawytscha*, from the release of fingerlings having 7.9% lipid content than from those having 4.1%.

Condition factor of juveniles at Mactaquac remains above 1.0 during January, February, and most of March, decreasing to <1.0 during April. Liberation to the Saint John River before the second half of April could result in lower rates of adult return, as a proportion of the juveniles are nonmigratory at that time. Wagner (1968) observed that rate of returning adults from the release of steelhead, *Salmo gairdneri*, smolts in the Alsea River, Oregon, during April was greater than that from February and March releases. The early liberation groups had higher K values than the April group, and the lower rates of return were attributed to greater proportions of nonmigrants, resulting in higher rates of freshwater postliberation mortality (Wagner 1968).

The influence of timing of smolt release on subsequent adult return was first investigated at Mactaquac during 1975 when two groups of 6000 smolts were forced from the station on May 2 and two similar groups on May 26. Returns of 1-sea-winter salmon from the early and late release groups were equal, suggesting that liberation dates that maximize rate of return are relatively flexible within the month of May. The flexibility in release date during May at Mactaquac is presumably attributable, in part, to the relatively small lipid losses ($\leq$10%) during that period and to the occurrence of migratory activity throughout the month. Condition factor of smolts at Mactaquac increases during the late-May to early-June period coincident with cessation of migratory activity and attainment of the postsmolt condition, suggesting that June liberation could result in lower adult return.

At Mersey, condition decreased until the end of May when the last groups of juveniles were released. Presumably, K values would have increased during June as at Mactaquac marking the cessation of migratory activity. In contrast, lipid content of smolts at Mersey decreased at 3 times the rate observed for Mactaquac smolts and migratory activity was apparent 3 wk earlier. Water temperatures at Mersey increase to 10°C by the first of May and 15°C at the end of that month, whereas these temperatures are not attained at Mactaquac until late May and mid-June, respectively. Although day length was slightly longer at Mersey (+10 min on May 1), the advanced temperature cycle at that location is probably responsible for the earlier onset and extended period of migratory activity that results in a greater rate of lipid utilization. Because of the rate of lipid loss among smolts at Mersey, the period of release at that location is less flexible than that recommended for Mactaquac smolts. Judging from the onset of migratory activity and from changes in K values and lipid content, the late-April to early-May period appears appropriate for smolt liberation from Mersey to the LaHave River.

Observations of the downstream movement of smolts released in the LaHave River during the last week of April 1971 indicated that fish began migration before mid-May. Adult return rates from the liberation of yearling smolts in this river during the late-May to early-June period (1972–74) were markedly lower than those from the release of 2-yr-old smolts during early May. Our study indicates that transformation is synchronous for juveniles >14 cm and that smolts of that size be liberated in southwest Nova Scotia between late April and early May. The lower returns resulting from yearling liberation cannot be attributed to failure of development of the marine osmoregulatory mechanisms of the younger smolts or totally to their smaller size, but rather to the late period of release.

Changes in lipid and moisture content and K values occurring during transformation at Mactaquac and Mersey were greater for larger than for smaller smolts, suggesting that energy expenditure associated with transformation may be size specific and that migratory activity of larger smolts may be greater. Accordingly, larger wild smolts have shown migratory activity earlier than those smaller (Saunders and Allen 1967; Österdahl 1969). In contrast, lipid and moisture content and K values of juveniles $\leq$12 cm changed little during the spring, indicating that the majority of individuals of that size did not undergo transformation.

In summary, the timing and magnitude of migratory activity and of the changes associated with parr–smolt transformation vary at different Maritime hatcheries because smolts are tuned to environmental conditions unique to their particular location. Danger exists in the practice of rearing smolts at one location then transporting them during their migratory phase to distant release sites where the temperature cycle is different. Transportation of juveniles to distant release ponds when water temperatures are low and the process of transformation not well advanced may be a solution. Smolts could then be held until in phase with the light and thermal regimes in their new location. It is important to release smolts at the beginning of their migratory period to ensure higher energy reserves at that time.

Identification of a minimum size for seawater adaptation and parr–smolt transformation will facilitate grading practices at Maritime hatcheries. Parr <12–13 cm could be liberated in fresh water during late fall assuming they will not undergo transformation the following spring. This will reduce pond densities, possibly resulting in an improvement in quality of the larger juveniles destined to be smolts.

Finally, the 1- and larger 2-yr-old smolts adapted to

seawater equally well, indicating the desirability of yearling smolt programs where temperature regimes are conducive to rapid growth. Not only are increases in production possible with this type of program, but adult returns from the release of yearling smolts can be equal to returns from the release of the larger 2-yr-old individuals (Peterson 1973; Isaksson 1976).

Acknowledgments

We thank the staff of the Mactaquac and Mersey hatcheries particularly managers J. McAskill and T. Goff for their cooperation. We are grateful to T. Carey, E. B. Henderson, B. Riddell, F. W. H. Beamish, J. Leatherland, and especially P. F. Lett and R. L. Saunders for their constructive criticisms of the manuscript.

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Distribution and Morphology of the Rodlet Cell in Fish

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MORRISON, C. M., AND P. H. ODENSE. 1978. Distribution and morphology of the rodlet cell in fish. J. Fish. Res. Board Can. 35: 101–116.

A short review of the work done on the rodlet cell is presented. The morphology and distribution of rodlet cells in several species of fish, primarily marine, is described and compared with that reported by other authors, mainly in freshwater fish. The morphology in freshwater and marine fish is similar, but variations are present in different tissues in the same fish. The rodlet cell was found in a wide range of tissues in most of the fish studied. It usually occurs in epithelia, with the apex facing the lumen. The possible function of the rodlet cell is discussed.

Key words: rodlet cell, fish, morphology, ultrastructure

MORRISON, C. M., AND P. H. ODENSE. 1978. Distribution and morphology of the rodlet cell in fish. J. Fish. Res. Board Can. 35: 101–116.

Les auteurs passent brièvement en revue les travaux effectués sur les bâtonnets. Ils décrivent la morphologie et la distribution des bâtonnets chez plusieurs espèces de poissons, surtout de mer. Ils les comparent avec les observations d'autres auteurs, en grande partie sur des poissons d'eau douce. La morphologie est semblable chez les poissons d'eau douce et les poissons de mer, mais il existe des variations dans différents tissus d'un même poisson. On trouve des bâtonnets dans une gamme étendue de tissus chez la plupart des poissons étudiés. La cellule se rencontre ordinairement dans l'épithélium, l'apex donnant sur la lumière. On analyse la fonction possible de la cellule en bâtonnet.

Received February 22, 1977
Accepted September 1, 1977

Reçu le 22 février 1977
Accepté le 1 septembre 1977

THE rodlet cell *Rhabdospora thelohani* or Stäbchendrüsenzellen is probably familiar to most fish histologists and has been frequently described in the literature. The authors of studies on the rodlet cell, and the species of fish and tissues studied, are listed in Table 1. The cell may be a parasite (Bannister 1965a, b, 1966; Bernt 1938; Dawe et al. 1960; Flood et al. 1975; Hale 1965; Iwai 1968; Labbé 1896; Laguesse 1895, 1906; Laibach 1937; Mourier 1970; Thelohan 1892) or a normal cell (Al-Hussaini 1949a, b; Bishop and Odense 1966; Bühler 1930; Bullock 1963, 1967; Catton 1951; Desser and Lester 1975; Dornesco and Santa 1963; Duthie 1939; Eberl-Rothe 1952; Fernhead and Fabian 1971; Kato 1935; Klust 1940; Leino 1974; Plehn 1906a, b; Smallwood and Smallwood 1931; Vickers 1962; Wilson and Westerman 1967). The literature on the rodlet cell is extensive, and covers a wide range of tissues, so we thought it would be useful at this time to summarize the present state of knowledge of the rodlet cell in a short review.

We have often found rodlet cells in tissues of marine fish during the course of various studies. Relatively few authors have investigated the distribution and morphology of the rodlet cell in marine fish (Duthie 1939; Laguesse 1895, 1906; Kato 1935; Iwai 1968; Fernhead and Fabian 1971), so we present a summary of our findings on rodlet cells in various tissues in marine fish, and compare their occurrence and appearance with that described by other authors.

Printed in Canada (J4738)
Imprimé au Canada (J4738)

Review

The rodlet cell is pear-shaped with a distinct capsule. It has a nucleus at the wider end, and longitudinally oriented rodlets. It is often found in epithelia of the tissues listed in Table 1, oriented parallel to the epithelial cells with the basal end containing the nucleus distal to the lumen. In situations such as connective tissue, however, no special orientation has been described. Many authors favor the idea that the rodlet cell is a parasite because it is found in so many different tissues, and its abundance varies. Proponents of the parasite theory originally assumed that the cell was a sporozoan, because it had a distinct capsule, and assumed that the rodlets were sporozoites (Laguesse 1895, 1906; Thelohan 1892). Laguesse named the rodlet cell *Rhabdospora thelohani* after Thelohan, at the suggestion of Henneguy, because he first described it. However, the staining reactions of the rodlets (Table 2) were not found to be typical of nuclei. The rodlets stained with iron hemotoxylin (Vickers 1962); but

TABLE 1. Occurrence of rodlet cells in fish. B brain, BA bulbus arteriosus, BV blood vessels, CT subcutaneous connective tissue, D bile or pancreatic ducts, E eye, G gonad, Gi gills, I intestine, K kidney, L liver, M mesentery, NE nasal epithelium, P pancreas, PC pyloric cecum, PE pharyngeal epithelium, R rectum, S stomach.

			Tissues (X present) (X* tentative)																
Family	Common name	Species	PE	S	I	PC	R	K	G	P	L	D	NE	Gi	B	E	BA	BV	M	CT
Cyprinidae	Goldfish	*Carassius auratus*[13,21,26]			X							X	X	X			X			
	European cruciancarp	*C. carassius*[27]											X							
	Carp	*Cyprinus carpio*[1,9,11,18,23,24,25]			X		X	X						X			X	X		
	Tench	*Tinca tinca*[23,25]						X						X			X			
	Bleak	*Alburnus alburnus*[25]																		X
	River shiner	*Notropis blennius*[21]			X			X						X			X		X	
	Redfish shiner	*N. umbratilis*[21]			X			X						X			X		X	
	Swordtail	*Xiphophorus helleri*[21]			X									X			X		X	
	Barbel	*Barbus fluviatilis*[23]												X				X		
	Minnow	*Phoxinus phoxinus*[2]	X					X	X		X		X		X	X				
	Dace sp.	*Phoxinus* sp.[25]							X											
	Roach	*Rutilus rutilus*[1,6]			X									X					X	
Percidae	Perch	*Perca fluviatilis*[6,25]			X	X								X					X	
	Yellow perch	*P. flavescens*[21]			X			X						X						
	Walleye	*Stizostedion vitreum vitreum*[21]			X			X						X						
Poeciliidae	Guppy	*Poecilia reticulata*[14]		X	X					X	X									
	Mosquito fish	*Gambusia affinis*[5]			X			X				X								
Salmonidae	Brook trout	*Salvelinus fontinalis*[5]			X															
	Rainbow trout	*Salmo gairdneri*[5]			X														X	
	Brown trout	*S. trutta*[5,6]			X									X					X	
	Lake trout	*Salvelinus namaycush*[21]			X			X						X						
	Atlantic salmon	*Salmo salar*[5]			X															
Anguillidae	American eel	*Anguilla rostrata*[5]			X															
	European eel	*A. anguilla*[3,20]			X								X							
Catostomidae	White sucker	*Catostomus commersoni*[5,7,8]			X			X				X		X				X		
Gobiidae	Gudgeon	*Gobio gobio*[1]			X															
Triglidae	Tub gurnard	*Trigla lucerna*[10]			X			X											X	
	Red gurnard	*Aspitrigla cuculus*[10]			X			X											X	
Labridae	Ballam wrasse	*Labrus bergylta*[10]			X			X											X	
	Cuckoo wrasse	*L. mixtus*[10]			X			X											X	
	Corkwing	*Crenilabrus melops*[10]			X			X											X	
Stromateidae	Man of war fish	*Nomeus gronovii*[17]		X	X	X														
		Stromateus fiatola[4]		X																
Esocidae	Northern pike	*Esox lucius*[21]			X			X*						X						
Centrarchidae	Smallmouth bass	*Micropterus dolomieui*[21]			X			X						X					X*	
	Rock bass	*Ambloplite srupestris*[21]			X															
Gadidae	Burbot	*Lota lota*[21]			X									X						
Cottidae	European bullhead	*Cottus gobio*[23]						X												
Scorpaenidae	Black rockfish	*Sebastes inermis*[16]				X														
Gasterosteidae	Threespine stickleback	*Gasterosteus aculeatus*[14,22,25]		X	X			X												
	Brook stickleback	*Culaea inconstans*[15]						X		X	X									
Monodactylidae	Silver batfish	*Monodactylus argenteus*[12]												X						

[1]Al-Hussaini (1949a, b). [2]Bannister (1965a, b, 1966). [3]Bernt (1938). [4]Bühler (1930). [5]Bullock (1963, 1967). [6]Catton (1951). [7]Dawe et al. (1960). [8]Desser and Lester (1975). [9]Dornesco and Santa (1963). [10]Duthie (1939). [11]Eberl-Rothe (1952). [12]Fernhead and Fabian (1971). [13]Flood et al. (1975). [14]Hale (1965). [15]Hess (1918). [16]Iwai (1968). [17]Kato (1935). [18]Klust (1940). [19]Laguesse (1895, 1906). [20]Laibach (1937). [21]Leino (1974). [22]Mourier (1970). [23]Plehn (1906a). [24]Smallwood and Smallwood (1931). [25]Thelohan (1892). [26]Vickers (1962). [27]Wilson and Westerman (1967).

TABLE 2. Summary of staining properties of rodlets recorded in the literature.

Author	Species	Stain	Staining reaction of rodlets	
Bannister (1966)	*Phoxinus phoxinus*	Giemsa	+	
		Hematoxylin	+	(cortex only)
		Hemalum	+	(cortex only)
		Feulgen	−	
		PAS	+	
		Lugol's I.	−	
Bullock (1963)	*Salvelinus fontinalis*	PAS	+	(still + after salivary digestion; − after sodium sulfate blockage)
	Salmo salar	Fast green	+	
	S. gairdneri	Eosin	+	
	S. trutta	Thionin	−	
		Azure C	−	
		Mallory's phospho-tungstic acid hematoxylin	−	
		Baker's acid hematin	−	
		Baker's modification of Sakaguchi's arginine technique	−	
		Gentian violet	−	
		Rinehart	−	
		Abul-Kaj	−	
		Alkaline phosphatase	−	
Bullock (1967)	*Gambusia affinis*	Fast green	+	
		Anilin blue	+	
		PAS	+	
		Aldehyde fuchsin	+	
Fernhead and Fabian (1971)	*Monodactylus argenteus*	Urates	−	
		Collagen	−	
		Calcium salts	−	
		Sodium	−	
Iwai (1968)	*Sebastes inermis*	Eosin	+	(central axis slightly stained with hematoxylin)
		Fast green	+	
Mourier (1970)	*Gasterosteus aculeatus*	PAS	+	
Plehn (1906a)	*Barbus fluviatilis*	Hemalum	+	(very light)
	Cyprinus carpio	Carmine	−	
	Tinca vulgaris	Safranin	−	
	Gobio gobio	Thionin	−	
		Bleu de Lyon	+	
		Delafield's hematoxylin	+	
		Mayer's muchemalum	−	
Thelohan (1892)	*Gasterosteus aculeatus*	Safranin	+	(more diffuse than nucleus)
	Perca fluviatilis			
	Cyprinus carpio			
	Tinca tinca			
	Phoxinus sp.,			
	Alburnus alburnus			

Author	Species	Stain	Beads	Strings
Vickers (1962)	*Carassius auratus*			
		Gram	+	+
		PAS	+	+
		Ald. fuchsin	+	+
		Azan	blue	red
		Iron hematoxylin	+	+
		Feulgen (nucleus +)	+	−
		Sudan black (60°C)	+	−
		Osmiophilia	+	+

when the specimen was stained with hematoxylin or hemalum, only the cortex of the rodlet was stained (Bannister 1966; Iwai 1968) or the staining was light (Plehn 1906a) or negative (Bullock 1963). The rodlets stained only lightly with safranin (Thelohan 1892). One author reported positive staining with Feulgen's technique (Vickers 1962), whereas another reported no staining (Bannister 1966). Perhaps Vicker's "beads" are not part of the rodlets. Positive staining with the periodic acid-Schiff (PAS) technique (Mourier 1970; Bullock 1963, 1967; Vickers 1962; Bannister 1966) and aldehyde fuchsin (Bullock 1967; Vickers 1962) led Bullock to suggest that the rodlets consist of a neutral or alkaline mucopolysaccharide. The ultrastructure of the rodlets does not look like that of typical nuclei, since the rodlets appear to be paracrystalline (Desser and Lester 1975). The most likely candidate for a parasitic nucleus is the basal nucleus of the rodlet cell (Bannister 1966). The rodlet cell appears to expel its rodlets as it reaches the surface of the epithelium, and this was assumed to be the stage at which sporozoites were released (Thelohan 1892). Apart from this, no other forms that could be different stages in the life cycle of a parasite have been described. The rodlet cell could be related to the sporozoan *Eimeria gadi* (Thelohan 1892), but certainly the ultrastructure of *E. gadi* (Odense and Logan 1976) is not similar to that of the rodlet cell.

The rodlet cell seems too widespread to be a parasite, which is usually restricted to certain tissues of a fish, and the same parasite would not normally be found in so many species of fish (Desser and Lester 1975). It has been found in very young fish (Leino 1974), when parasitic infection would be unlikely. The lack of different stages, the presence of a normal nucleus, and the more recent finding that the capsule appears to consist of filaments, possibly of a contractile nature (Leino 1974; Mourier 1970; Flood et al. 1975; Desser and Lester 1975), indicate that the rodlet cell could be a normal cell. Apical cell junctions are present between rodlet cells and adjoining cells (Desser and Lester 1975). Junctions have also been described between other regions of adjoining rodlet cells (Mourier 1970), and between rodlet cells and cells of the fish (Leino 1974). This would indicate that the rodlet cells are also part of the fish.

Seen under light microscopy the rodlet cells have a nucleus and capsule that are light staining and fairly normal in appearance until the cell reaches the lumen, when both become irregular in shape and dense staining (Thelohan 1892; Leino 1974). These differences in appearance could be caused by different stages of secretion of the cell.

Leino 1974 and Desser and Lester 1975 suggested that the cells secrete by contraction of the capsule. Pinocytotic vesicles and microtubules have been described in the capsule of the cell, especially near the base, and in the vesicles surrounding the rodlets (Leino 1974; Flood et al. 1975; Fernhead and Fabian 1971), and Fernhead and Fabian (1971) suggested that these could move materials into the cell.

A secretory cell would be expected to develop from the germinal epithelium, in which case early stages should be present. Stages in which the capsule was not as distinct as in the mature form, and rodlet formation had just started, have been described (Desser and Lester 1975; Leino 1974; Flood et al. 1975). The "crystalline inclusion phase of the preencased endothelium-associated cell" of Flood et al. has not been described by other authors. It looks similar to the eosinophil in the same fish (fig. 5–8, p. 80 in Andrew 1965). The appearance of the rodlet cell in so many different tissues and its variability in numbers makes the idea that it is related to blood cells attractive (Duthie 1939; Catton 1951); but this is unlikely unless the crystalline inclusion phase of Flood et al. really is a stage in the development of the rodlet cell, since the crystalloids found in fish leucocytes (Andrew 1965; Kelenyi and Nemeth 1969) have an appearance different from those making up the rodlets of the mature rodlet cell.

If the rodlet cell is a normal cell of fish, then the question of its function arises, especially since it is so widespread in different tissues. Authors have tended to associate the function of the rodlet cell with the particular structure they were studying. For example, those studying blood cells assumed that it was the discharging phase of a granulocyte (Duthie 1939; Catton 1951); those studying the digestive tract assumed that it was a secretory cell (Bishop and Odense 1966; Al-Hussaini 1949a, b); those studying the olfactory epithelium assumed it was a sensory or secretory cell (Wilson and Westerman 1967); and those studying osmoregulation in the gills assumed it had a salt-secreting function (Fernhead and Fabian 1971). The only common feature in its occurrence is that it appears to secrete into extracellular spaces; either the external environment or passages leading to the external environment as in the gills, nasal epithelium, intestine, bile and pancreatic ducts, or kidney; or into the body cavity such as the mesothelium, or into blood vessels. It is possible that in tissues such as brain, liver, pancreas, and gonad, the rodlet cells secrete their contents into blood vessels. In the liver, pancreas, and gonad they could also be secreting into the body cavity, or in the liver they could be secreting into branches of the bile duct, in the pancreas into branches of the pancreatic duct, and in the gonad into reproductive ducts. Rodlet cells could be involved with water and electrolyte balance, or have functions similar to those suggested for mucous cells (Leino 1974). Changes in numbers of rodlet cells have been found in response to changes in concentrations of cobaltous and manganous salts (Vickers 1962); and changes in numbers and in appearance have been described in response to changes in salinity (Fernhead and Fabian 1971), so they may be formed in response to certain extracellular conditions, which could explain why their numbers vary. We are attempting to elucidate the function of these cells by investigating the effects

of changes in the concentration of different ions in the environment on the distribution and appearance of these cells in different tissues.

Methods

The tissues and species of fish examined are listed in Table 3. Most of the fish are marine. Some euryhaline fish (rainbow and brook trout and sturgeon) and freshwater fish (guppies and zebra fish) were also sampled to ensure that any differences found between freshwater and marine fish were not due to differences in methods of preparation. The spring peeper and red-spotted newt were examined to see whether the rodlet cell was present in Amphibia. The cod was the marine fish studied most extensively, since it was the easiest species to obtain and maintain. The rodlet cells were most numerous and easy to identify in the intestine, kidney, and pyloric ceca of cod, so these tissues were normally studied in other species.

For light microscopy tissues were fixed in 10% formalin, or Lison's Gendre fluid for Best's glycogen method (Pearse 1968). The sections were usually stained in hematoxylin and eosin, but some were stained with the Fast Green modification of the Van Gieson Stain (Biological Stain Commission 1960). The following histochemical tests were carried out to study the nature of the rodlet cell: the periodic acid-Schiff, alcian blue, and aldehyde fuschin tests, an azo-coupling method for argentaffin granules (Humason 1972), the mercury bromophenol blue method for protein, the DMAB-nitrite method for tryptophan, Best's method for glycogen, sudan black in propylene glycol for lipid, and a method for lactic dehydrogenase (Pearse 1968). The methylene blue extinction test (Pearse 1968) was carried out at pH 3.7–8 on sections of cod ileum to cover the range at which mucous cells show metachromasia, to ascertain whether rodlet cells also contain mucus. Rodlet cells from various species were measured in the microscope using a micrometer eyepiece calibrated with a stage micrometer.

Pieces of cod kidney were excised under aseptic conditions and left in a trypsin digestion medium overnight. Photomicrographs of the freed rodlet cells were taken under phase illumination. Specimens of cod kidney, intestine and pyloric cecum, speckled trout pyloric cecum, American plaice intestine and pollock pyloric cecum were excised, fixed in phosphate-buffered glutaraldehyde, then osmium tetroxide, dehydrated in acetone or alcohol, passed through propylene oxide if alcohol was used, then embedded using the Durcupan ACM kit. They were sectioned, double-

TABLE 3. Distribution of rodlet cell in 18 marine and 4 freshwater species of fish. See Table 1 for explanation of abbreviations.

Family	Common name	Species	No. specimens	Tissue	Rodlet cells (X present) (– absent)
Gadidae	Atlantic cod	*Gadus morhua*	40	K	X
			2	K	–
			3	NE	X
			1	NE	–
			1	I	X
			4	PC	X
			1	PC	–
			1	Ureter	–
			1	Bladder	–
			1	Testis	–
			1	Ovary	–
			1	Brain	–
			1	Liver	–
	Pollock	*Pollachius virens*	1	PC	X
			1	NE	X
			1	I	X
			1	K	–
	Haddock	*Melanogrammus aeglefinus*	2	I	X
			2	I	–
			2	PC	X
			1	PC	–
			2	K	–
			1	NE	–
	White hake	*Urophycis tenuis*	1	K	–
			1	PC	–
			1	NE	–
			1	I	–
Lophiidae	Goosefish	*Lophius americanus*	1	I	?
			1	PC	–
Cottidae	Sea-raven	*Hemitripterus americanus*	2	I	X
			1	I	–
			1	PC	–
	Longhorned sculpin	*Myoxocephalus octodecemspinosus*	2	I	X
			2	PC	X

TABLE 3 (*Concluded*)

Family	Common name	Species	No. specimens	Tissue	Rodlet cells (X present) (– absent)
Labridae	Cunner	*Tautogolabrus adspersus*	1	I	X
			1	M	X
Congridae	Conger eel	*Conger oceanus*	2	I	X
Clupeidae	Atlantic herring	*Clupea harengus harengus*	2	I	X
			1	PC	X
			1	Pancreas	–
	Alewife	*Alosa pseudoharengus*	2	I	–
Anarhichadidae	Atlantic wolffish	*Anarhichas lupus*	2	I	–
Pleuronectidae	Witch flounder	*Glyptocephalus cynoglossus*	1	I	X
			1	PC	–
			1	Mesentery and CT round arteries	?
	American plaice	*Hippoglossoides platessoides*	1	I	X
			1	PC	X
	Winter flounder	*Pseudopleuronectes americanus*	2	I	–
			2	PC	X
	Yellowtail flounder	*Limanda ferruginea*	2	I	?
			2	PC	–
Salmonidae	Rainbow trout	*Salmo gairdneri*	2	PC	–
			1	PC	X
			1	NE	–
			3	K	–
			1	K	?
			1	Gill arch	–
			1	Gill arch	?
			4	I	–
			1	Spleen	–
			1	Gonad	–
	Brook trout	*Salvelinus fontinalis*	2	PC	–
			1	PC	X
			1	NE	–
			2	K	–
			1	K	?
			1	Gill arch	–
			4	I	X
			2	I	–
			1	Eye	–
			1	Heart	–
			1	Stomach	–
			1	♀ gonad	–
			1	Liver	–
Poeciliidae	Guppy	*Poecilia reticulata*	1	I	+
				CT under skin	?
Rajidae	Skate	*Raja ocellata*	2	Spiral value	–
Acipenseridae	Atlantic sturgeon	*Acipenser oxyrhynchus*	2	I	–
				Heart	–
				Gills	–
Cyprinidae	Zebra fish	*Brachydanio rerio*	1	I	+
				Connective tissue next to spinal cord	+
				Skin	?
				Pancreas, stomach	–
				Muscle and cartilage	–
				Eye and brain	–
				K	–
				Reproductive system	–
Amphibia	Spring peeper	*Hyla crucifer*	1	whole animal	–
	Red spotted newt	*Triturus vulgaris*	1	whole animal	–

TABLE 4. Staining properties of rodlet cells in 22 species of marine and freshwater fishes.

Stain	Species	Tissue	Rodlets	Capsule	Nucleus
Hematoxylin and eosin (Humason 1972)	All species with rodlet cells in Table 1	All tissues in Table 1	+ (pink)	+ (pink)	+ (purple)
Van Gieson (Biological Stain Commission 1960)	"	"	+ (Green and purple)	+ (Green)	+ (Green)
Mercuric bromophenol blue (Pearse 1968)	Cod	K	+	+	+
	Haddock	PC	+	+	+
	Pollock	PC	+	+	+
Periodic acid-Schiff (Humason 1972)	Cod	I	+	+	+
		PC	+	+	+
		K	+	+	+
	Haddock	PC	+ Weakly staining	+	+
	Herring	PC	+	+	+
	Sea-raven	I	+	+ Weakly staining	+ Weakly staining
	Longhorned sculpin	I	+	+	+
	Speckled trout	PC	+	+	+
	Conger eel	I	+ Medium staining	+	+
	American plaice	I	+	+	+
	Witch flounder	I	+ Strongly staining	+	+
	Sea perch	I	+	+	+
Alcian blue (Humason 1972)	Cod	K	−	−	−
	Haddock	K	−	−	−
		PC	−	−	−
	Pollock	K	−	−	−
		PC	−	−	−
DMAB-nitrite method for tryptophan (Pearse 1968)	Cod	I	+ Very weak	+ Very weak	+ Very weak
		PC	+	+	+
Aldehyde fuschin (Humason 1972)	Speckled trout	PC	+	−	−
	Haddock	PC	+ Very weak	−	−
	Herring	PC	+	−	−
	Conger eel	I	+	−	−
	Searaven	I	+	−	−
Best's glycogen (Pearse 1968)	Cod	I	−	−	−
		PC	−	−	−
Sudan black in propylene glycol (Pearse 1968)	Cod	I	−	−	−
		PC	−	−	−
Azo-coupling method for argentaffin granules (Humason 1972)	Cod	PC	−	−	−
Lactate dehydrogenase (Pearse 1968)	Cod	I	−	−	−
		PC	−	−	−
Heidenhain iron hematoxylin (Humason 1972)	Cod	K	+	+	+
	Speckled trout	PC	+	+	+

stained in 25% uranyl acetate in methanol (Stempack and Ward 1964) and Millonig's lead stain, and viewed and photographed in a Hitachi HS7S electron microscope.

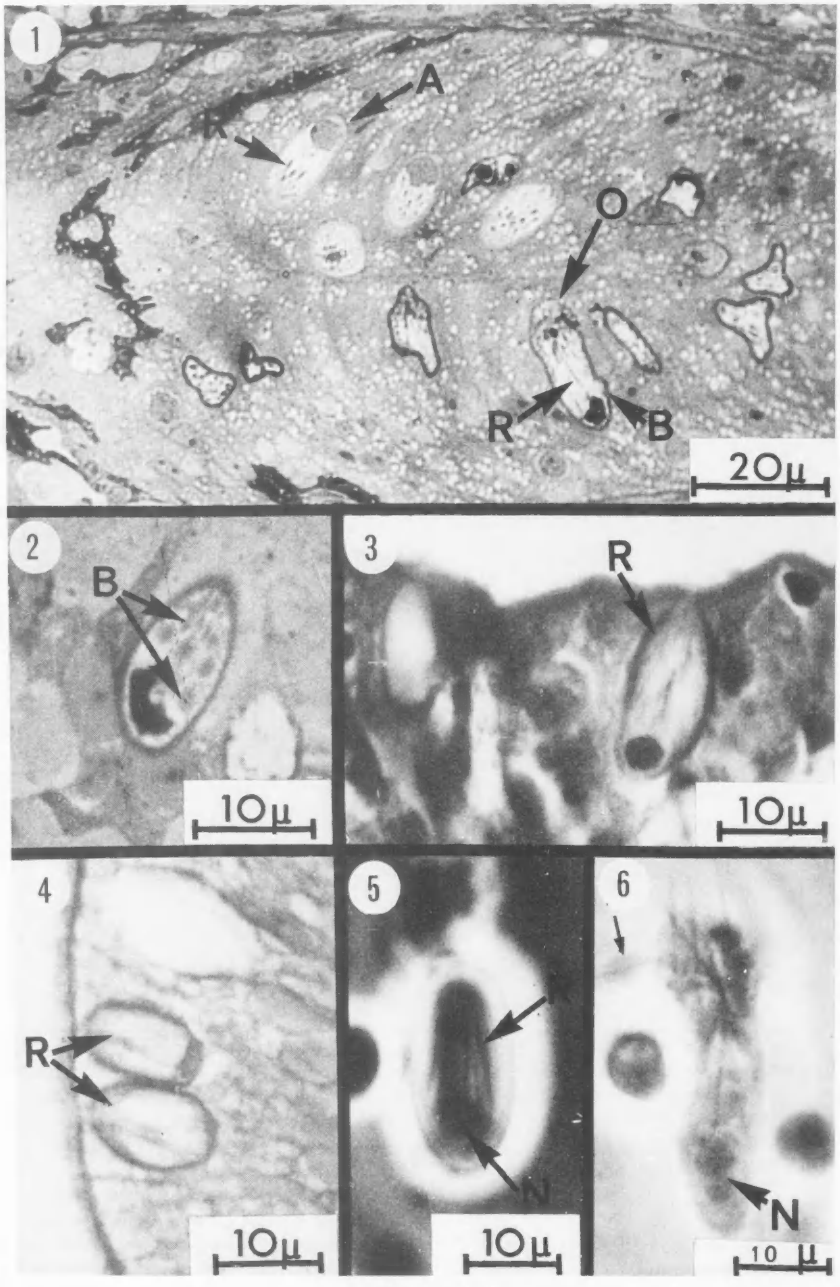
Observations

LIGHT MICROSCOPY

The results of staining methods and histochemical tests for the rodlet cell are summarized in Table 4. Rodlet cells were found to show most distinctly in sections stained using the Van Gieson method.

Rodlet cells usually occur in epithelia, oriented parallel to the epithelial cells with the nucleus distal to the lumen (Fig. 1–4). The cells are usually oval, but some have an irregular, densely stained outline (Fig. 1), and sometimes the nucleus is irregular in shape and densely stained (Fig. 1 and 2). A thick capsule surrounds each cell, and refractile rods are present in the cytoplasm which are oriented more or less parallel to the long axis of the cell. In most species the rodlets

stain as thin, elongated structures, but in brook trout they are elliptical (Fig. 2). In the kidney most rodlet cells occur in the large collecting ducts. Rodlet cells were found in a wide range of species, and in different tissues (Table 3); but they were not necessarily found in all the tissues examined from one individual, in all individuals of the same species, or in all species of the same family.



FIGURES 1–6

The average length of the rodlet cells varies from about 7 μm in zebra fish to 19 μm in witch flounder. Individuals of the same species have a fairly constant size of rodlet cell, but the size is not constant in different species in the same family.

Rodlet cells were present in most cod, regardless of size, sex, and nutritional state. Eighteen juvenile cod varying from 27 to 129 g all had rodlet cells in the kidney, although the number of these varied greatly. Rodlet cells were found in the kidneys of four adult cod that were fed herring and liver for 3 wk and in three of four adult cod starved for at least 3 wk.

During the methylene blue extinction test metachromasia was demonstrated in mucous cells from pH 4.5 to 7.3; but no metachromasia was demonstrated in the rodlet cells. The nuclei of the rodlet cells were blue at all pH values tested, but became deeper blue at more alkaline pH. The rodlets were colorless at very acidic pH, but started to appear blue at pH 5.3, while remaining cytoplasm started to appear blue at pH 7.

Rodlet cells are easily recognized in tissue separated by trypsinizing. Intact rodlet cells (Fig. 5) are oval with a nucleus at one end. The rodlets are arranged like the ribs of an umbrella with the open ends around the nucleus, coming together at the end of the cell opposite the nucleus. In some rodlet cells (Fig. 6) the rodlets appear to have ruptured the cell at the end opposite to the nucleus. The rodlets remain intact outside the cell and oval globules of dense material are present around part of each rodlet. The outline of the ruptured cell is irregular, and the nucleus elongated, irregular in shape, and dense-staining.

Electron Microscopy

The rodlet cells had a similar appearance to those described by other authors and had the same basic structure in all species examined (Fig. 7–9).

Rodlet cells from different species vary in the way the electron-dense contents of the vesicles are distributed. The contents of the vesicles of rodlet cells from the pyloric ceca of the cod are usually concentrated towards the periphery, and the contents of the smaller vesicles are usually more electron-dense (Fig. 7 and 10), whereas rodlet cells from the cod kidney have vesicles with denser contents and membranous inclusions. The rodlet cells from the pyloric ceca of pollock (Fig. 8) and American plaice have vesicles with uniformly dense contents, and those of the brook trout (Fig. 9) have vesicles with a dense zone, separated from the rodlet and the membrane surrounding the vesicles by a lighter zone. Membrane-bound bodies are more commonly seen in the vesicles of the trout than in the marine species; and these vesicles occur at different levels in the cell, whereas those of the cod are mostly in a perinuclear position. A periodicity is sometimes visible in the rodlets of brook trout, but cannot be seen in cod. The extruded contents from the rodlet cell of the pollock include several intact rodlets surrounded by membrane-bound vesicles, and a rodlet can be seen passing from the rodlet cell into the lumen (Fig. 8).

The rodlets are approximately square in cross section, and the diameters of the vesicles vary (Fig. 10). The contents of each vesicle are often more concentrated toward its periphery, and the contents of the smaller vesicles are usually more electron-dense. At the apex of each rodlet the vesicle appears to be incomplete, so that the contents of the vesicle are continuous with the rest of the cell cytoplasm (Fig. 11). Sometimes the dense contents of the vesicles appear to be concentrating around the rods in this part of the cell. Endoplasmic reticulum is present: it is usually smooth but sometimes has a few attached ribosomes. The Golgi apparatus has some inflated cisternae, and is surrounded by small vacuoles.

When rodlet cells are cut in cross section, their outline may be round or irregular (Fig. 12). The contents

FIG. 1. Light micrograph of Durcupan ACM section of pyloric cecum of cod. Rodlet cells are oriented with their apex towards the lumen of the tubular gland, except for some that have been sectioned transversely or obliquely and may open into the gland out of the plane of section. Some cells have an oval capsule and rounded nucleus and stain moderately densely (A). Others have an irregular capsule and an irregularly shaped nucleus and stain very densely (B). Rodlets (R) are visible. Apical organelles (O) appear to be expelled from rodlet cell B. FIG. 2. Light micrograph of Durcupan ACM section of pyloric cecum of speckled trout. Capsule of oval cell is stained, and irregular nucleus is densely stained. Elliptical bodies (B) occur in apical and basal parts of cytoplasm. FIG. 3. Light micrograph of paraffin section of pyloric cecum of American plaice stained with hematoxylin and eosin. A rodlet cell (R) can be seen at the luminal surface of the epithelium. The small, round nucleus, capsule, and rodlets are visible. This rodlet cell is larger than those shown in other species. FIG. 4. Light micrograph of paraffin section of intestine of long-horned sculpin stained with Van Gieson's stain. Two rodlet cells (R) are visible; one has a basal nucleus. FIG. 5. Phase micrograph of trypsinized preparation of cod kidney. Nucleus (N) and rodlets (R) of a rodlet cell can be seen. Rodlets extend from periphery of nucleus and converge at opposite end of the cell to the nucleus. Outline of cell is oval, similar in shape to rodlet cell A in Fig. 1. FIG. 6. Phase micrograph of trypsinized preparation of cod kidney. Rodlets appear to have pierced the rodlet cell at opposite end to nucleus. Rodlets remain intact outside cell, and an oval dense body is present round each rodlet (arrow). Outline of cell is fusiform and irregular, and nucleus (N) is elongated and dark, like rodlet cell B in Fig. 1.

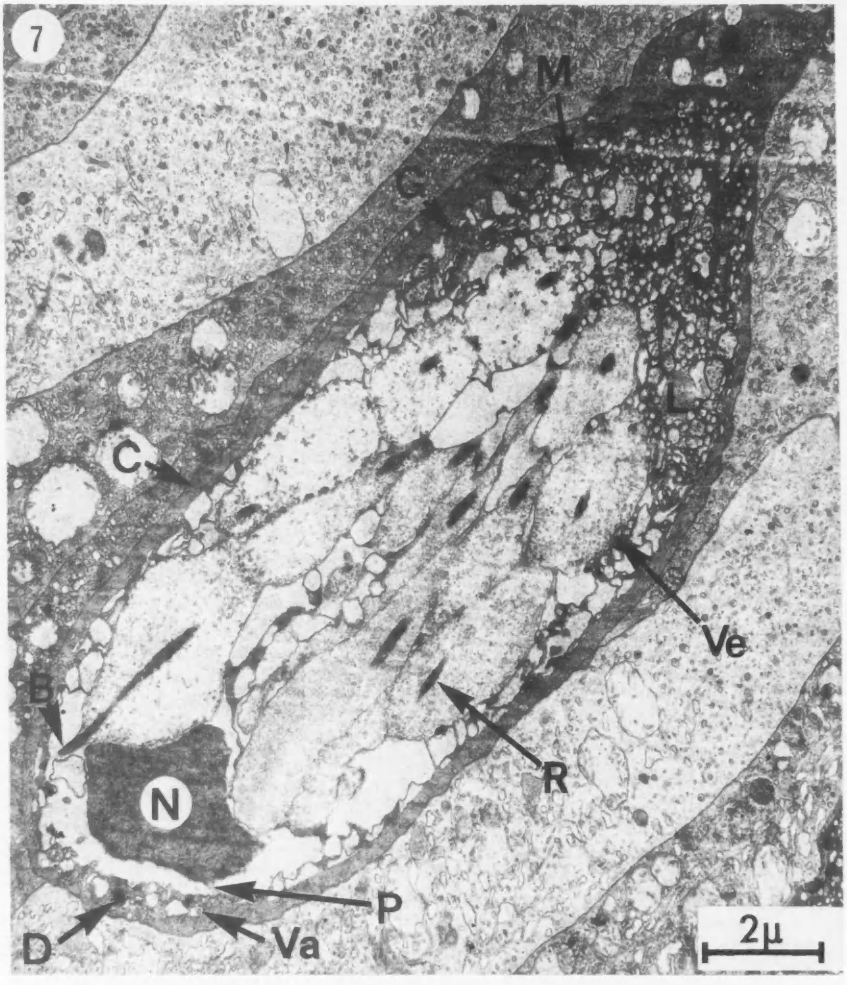


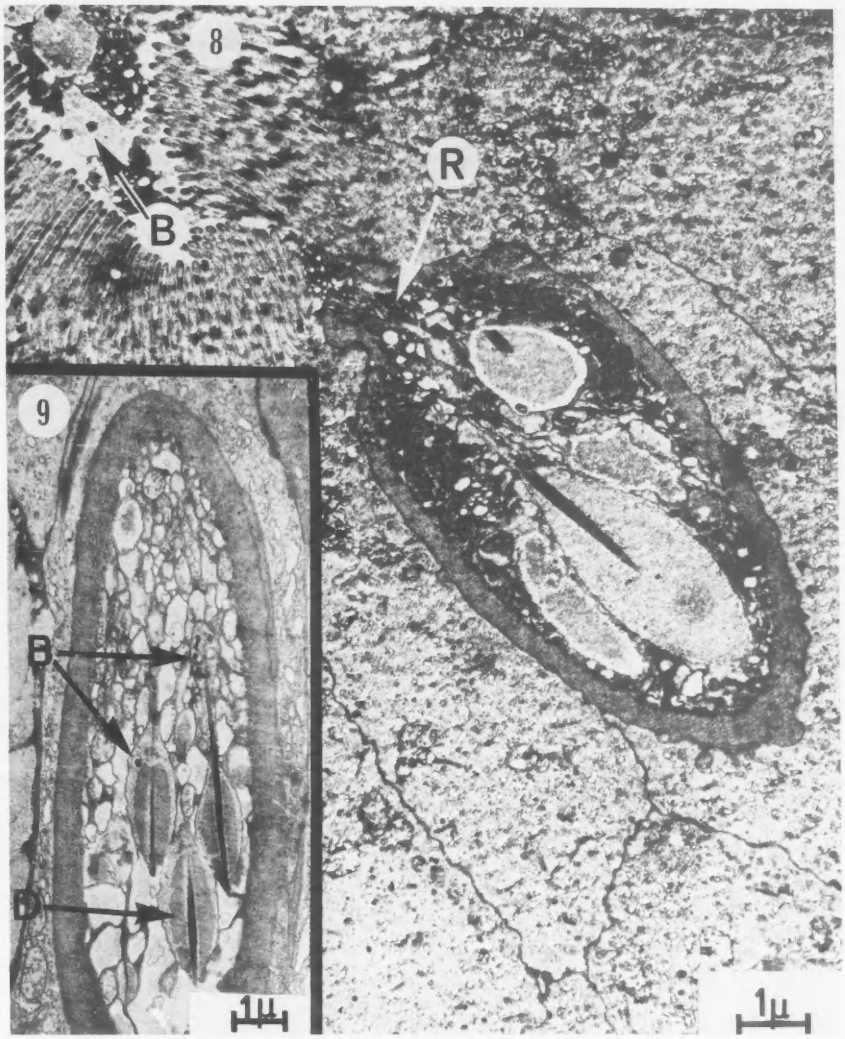
FIG. 7. Electron micrograph of longitudinal section of rodlet cell from pyloric cecum of cod. Rodlet cell has an irregular, densely stained nucleus (N) at the basal end of the cell. Rodlets (R) are very electron-dense, and oriented along the longitudinal axis of the cell, converging towards apical end. Nuclear end of each rodlet is surrounded by a membrane-bound vesicle (Ve) containing some electron-dense material; this vesicle becomes narrower round the rodlet towards apical part of cell and its contents become denser. Many small vacuoles and mitochondria (M) are present at apical end of cell. Under the cell membrane is a fibrous capsule (C), which is narrower at the apical end of the cell. The nuclear envelope is expanded, but nuclear pores (P) are still present. A dense body, probably a lysosome (L) and a Golgi apparatus (G) are evident. The basal part of a rodlet (B) appears to be attached to the membrane surrounding its vesicle. The capsule becomes irregular in shape at the basal end, and contains vacuoles (Va), some with membranous contents, and dense bodies (D).

of the round cells are like those of regular rodlet cells, but vesicles appear to break down and the rodlets become less evident as the capsule becomes more irregular. Filaments are less evident in the irregular capsule, which is also more electron-dense.

Early stages similar to those seen by other authors were found in cod, pollock, and American plaice.

Discussion

The appearance of the rodlet cell in marine fish resembles that described by other authors for freshwater fish. The distribution of the rodlet cell is similar, except we did not find it in gonads, nervous tissue, blood vessels, liver, pancreas, eye, or stomach. We confirm



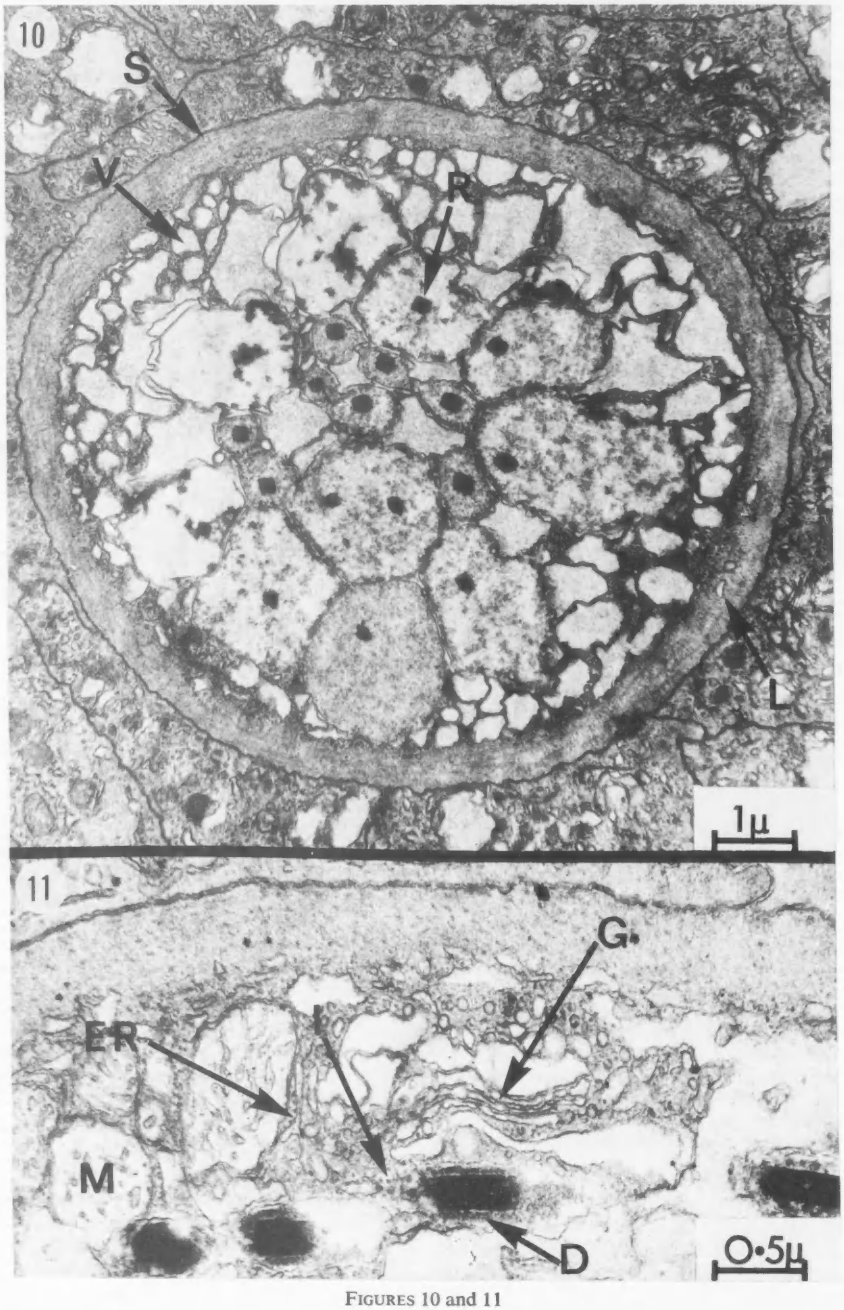
FIGURES 8 and 9

FIG. 8. Electron micrograph of longitudinal section of rodlet cell from pyloric cecum of pollock. Fibrous capsule of rodlet cell is open at apical end, and shows blebbing at basal end. A rodlet (R) was fixed as it passed through the opening, and round bodies (B) which appear to be vesicles containing rodlets are present in the lumen. FIG. 9. Electron micrograph of longitudinal section of rodlet cell from pyloric cecum of brook trout. Rodlets surrounded by vesicles occur at different levels in the cell. A zone of dense material (D) is present round each rodlet, and a relatively clear zone is present immediately adjacent to the rodlet and beneath the membrane of the vesicle surrounding the rodlet. Membrane-bound bodies (B) with electron-dense inclusions are present towards the apical ends of vesicles surrounding rodlets.

the findings of most other workers that the nucleus of the rodlet cell stains with typical nuclear stains such as hematoxylin, and Feulgen's test for DNA, whereas the rodlets do not; so it seems unlikely that the rodlets are nuclei of parasites.

The rodlets stain positively with Heidenhain's iron hematoxylin, mercury bromophenol blue, and the DMAB-nitrite method for tryptophan, indicating that they contain protein. We found, unlike other authors, that staining with the PAS test was variable, indicating that the rodlets contain varying amounts of polysaccharides. The rodlets did not stain with alcian blue and

acid fuchsin, so the polysaccharide is not acidic, and did not stain with Best's method for glycogen, so the polysaccharide is not glycogen. Tests for lipid and argentaffin granules were also negative. The methylene blue extinction test showed that metachromasia was not present in the rodlets, again indicating that acid mucopolysaccharide is not present (Pearse 1968). We obtained no staining for lipid, whereas the rodlets of



FIGURES 10 and 11

goldfish were reported as staining with sudan black indicating the presence of fat (Table 2, Vickers 1962). Otherwise the results of other authors are compatible with ours. These tests indicate that the rodlets are protein with varying amounts of attached sugars, probably in the form of a neutral glycoprotein. The staining reactions are not comparable with those of goblet cells, which contain acid mucopolysaccharides as well. It therefore seems unlikely that the rodlet cell is a different stage of a goblet cell, as suggested by Al-Hussaini (1949b); but if the rodlet cell is secreting glycoprotein, this secretion could have functions similar to those suggested for goblet cells (Leino 1974).

The rodlet cell varies in degree of staining with the PAS technique and in appearance in different species of fish. For example, with routine hematoxylin and eosin staining the rodlet cells in brook trout have a distinct capsule, but the rodlets are poorly stained; and the rodlet cells in flatfish are generally large with an indistinct capsule and are difficult to differentiate from surrounding cells in the gut epithelium. The rodlet cells of the long-horned sculpin, however, are easy to identify because the capsule and rodlets stain distinctly. The differences also extend to the ultrastructure of the cell. For example, the rodlets of cod usually extend from a perinuclear position to the apex of the rodlet. In the brook trout, however, they occur at different levels in the cell. Also, the density of the contents of the vesicles surrounding the rodlets looks similar to that described in other primarily freshwater fish (Mourier 1970; Flood et al. 1975; Desser and Lester 1975), whereas the contents in cod are less uniformly dense. The form of rodlet cell found in cod kidney is more similar to that found in brook trout than that from cod intestine, in that the contents of the vesicles are denser and membranous inclusions are present. Presumably, alterations in appearance are related to the functional state of the rodlet cell, and indicate that the cell is not a parasite, since a parasite such as *E. gadi* has the same appearance in the different gadoids in which it occurs (Fiebiger 1913).

Our study showed the presence of the rodlet cell in various tissues of a wide spectrum of marine fish, an exception being the elasmobranchs. However, the number of cells seen varied considerably in different tissues and in different individuals of the same species. This agrees generally with other authors (Table 1).

In some species only one rodlet cell was positively identified in an entire section so it is likely that we have missed rodlet cells in some species or tissues. In any case, our observations, with those of other workers, show that the rodlet cell is widespread, possibly ubiquitous. It varies in numbers more than one would expect from a normal cell such as a goblet cell, but not from a cell such as a granulocyte.

The arrangement of organelles is well polarized in the rodlet cell, and the cell is usually oriented in epithelia with its nucleus basal, and many mitochondria and vacuoles at the apex of the cell. We found, unlike Bannister (1966), that the mitochondria were similar to those in surrounding cells unless they were near the lumen of extruding cells, in which case they were rounded off. Ribosomes do not seem to be attached to the membranes of the vacuoles; presumably these are dilated cisternae of smooth endoplasmic reticulum. The arrangement of rodlets is especially well seen in the living intact cell (Fig. 5).

The arrangement of the membrane-bound vesicles containing rodlets in the cell is shown in Fig. 13. The apex of the vesicle appears to be continuous with the apical cytoplasm where the other cell organelles are concentrated, and condensation of material seems to occur next to the rodlets near the apex. This would indicate that the protein moiety of the glycoprotein is manufactured by the free ribosomes near the cell apex, the polysaccharide component on the cell membranes of this region, and the glycoprotein is then condensed onto the apex of the rodlet. The contents of the basal region of the vesicle are usually unstained or very lightly stained indicating that they are mainly aqueous. We found vacuoles in the capsules, especially at the nuclear end of the cell. Often the vacuoles have membranous contents, and similar vacuoles are sometimes seen in the vesicles round the rodlets, so maybe materials are transported in these vacuoles across the cell capsule to the rodlets.

The early stages of the rodlet cells show lack of polarization of the cell organelles. Possibly in the next stage the mitochondria, ribosomes and Golgi apparatus move to the apex so that material is added to

FIG. 10. Electron micrograph of transverse section of rodlet cell from pyloric cecum of cod. Rodlets (R) are almost square in cross section, and membrane-bound vesicles round them vary in diameter. Density of contents of vesicles varies, but generally vesicles with a smaller diameter have denser contents. Many vacuoles (V) are present in the cytoplasm, and between them many granules, probably ribosomes. Under the cell membrane the fibrous envelope contains filaments about 5–15 nm wide oriented in a circular direction. Between these are small granules and a few large (L) and small (S) vacuoles. FIG. 11. Electron micrograph of oblique section of a rodlet cell from cod kidney. Dense material (D) appears to be accumulating around the rodlets which are surrounded by small vesicles and are therefore near the apex of the cell. Membranes limiting vesicles are incomplete (I). Many mitochondria (M) are present. There is a Golgi apparatus (G) and endoplasmic reticulum with some attached ribosomes (ER). These are surrounded by many free ribosomes and vacuoles. The filaments in the fibrous capsule are sectioned obliquely.

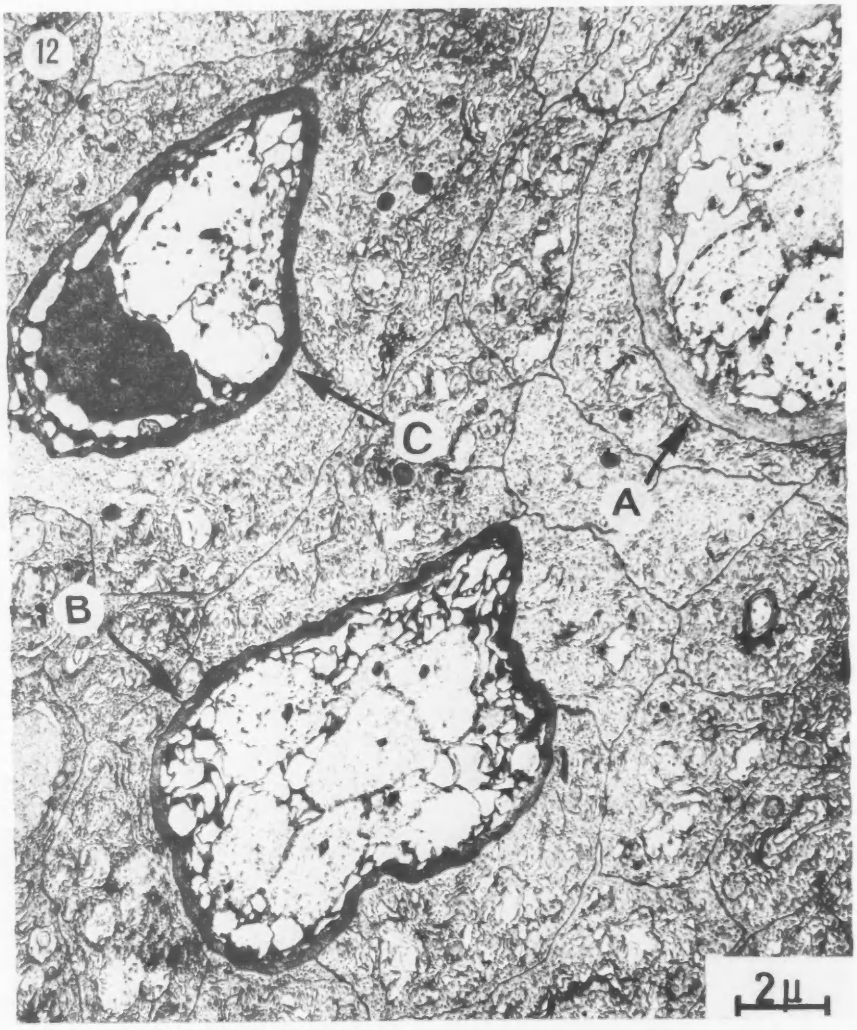
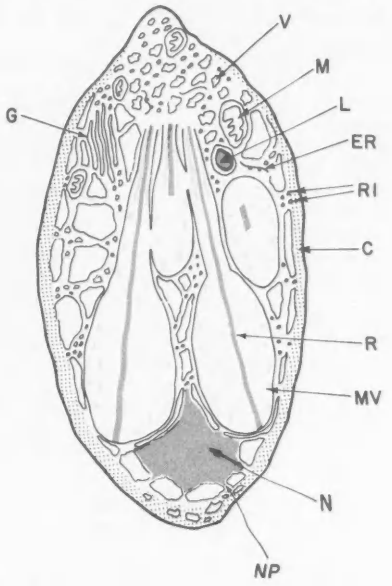


FIG. 12. Electron micrograph of transverse and oblique sections of rodlet cells from cod kidney. The capsule is least dense in one rodlet cell (A), moderately dense in another (B), and densest in a third (C). Well-formed rods, vesicles, and mitochondria can be seen in cell A, and the filaments are visible in the capsule. Vesicles are somewhat distorted in cell B, and filaments are no longer clearly seen in the capsule. The capsule is irregular in shape, whereas that of cell A is rounded. The nucleus seen in cell C is pycnotic, only one rod is visible, and the capsule is very densely stained and irregular in outline.

the apical ends of the rods as they become elongated.

The increase in density of the capsule as the rodlet cell secretes is visible in electron micrographs as well as light micrographs. Possibly the filaments become more disoriented and overlap as the capsule contracts and becomes irregular in shape.

In view of its wide occurrence and the presence of early and secreting stages, the rodlet cell is likely a normal cell of fish. The cell product is probably a neutral glycoprotein, which could have a similar function to the mucin secreted by goblet cells, but could be enzymic. The absence of rodlet cells in elasmobranchs, which use urea to maintain osmotic balance, supports the theory that they are involved in ion transport. The function of the capsule of the rodlet cell could be to expel the rodlets rapidly in response to a change in the



C	Capsule	M	Mitochondrion
ER	Endoplasmic reticulum	MV	Membrane-bound vesicle
G	Golgi apparatus	N	Nucleus
L	*Lysosome*	NP	*Nuclear pore*
R	Rodlet	RI	Ribosomes

V *Vacuole*

FIG. 13. Arrangement of membrane-bound vesicles containing rodlets in the cell.

extracellular medium, and the rodlet could be in a crystalloid form to provide a concentrated secretion or excretion.

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Geographic Variation in Atlantic Cod, *Gadus morhua*, off Eastern North America: A Biochemical Systematics Approach

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Cross, T. F., and R. H. Payne. 1978. Geographic variation in Atlantic cod, *Gadus morhua*, off eastern North America: a biochemical systematics approach. J. Fish. Res. Board Can. 35: 117–123.

Analysis of geographic variation in the frequencies of alleles at the *Tf* locus in Atlantic cod, *Gadus morhua*, indicates that it is possible to distinguish three major populations off eastern North America: (1) north of the Laurentian Channel, (2) south of the Laurentian Channel, and (3) Flemish Cap. The data also suggest that the north Laurentian stock may be further subdivided into a northern and southern component. The genetic distinction between the cod population on Flemish Cap and those on other areas of the continental shelf is confirmed by a significant difference in allele frequencies at the *PGI-2* locus. It is suggested that the cod population on Flemish Cap may have persisted in isolation since the last glaciation.

Key words: fish stock discrimination, transferrin, phosphoglucose isomerase, allele frequencies, genetic distance

Cross, T. F., and R. H. Payne. 1978. Geographic variation in Atlantic cod, *Gadus morhua*, off eastern North America: a biochemical systematics approach. J. Fish. Res. Board Can. 35: 117–123.

Une analyse de la variation géographique dans la fréquence des allèles au locus *Tf* chez la morue franche, *Gadus morhua*, indique qu'il est possible de distinguer trois populations majeures au large de l'Amérique du Nord orientale : (1) au nord du chenal Laurentien, (2) au sud du chenal Laurentien et (3) sur le Bonnet flamand. Les données suggèrent de plus que le stock du nord du chenal Laurentien peut se subdiviser en un composant septentrional et un composant méridional. Une différence significative dans la fréquence des allèles au locus *PGI-2* confirme la distinction génétique entre la population de morues du Bonnet flamand et celle des autres régions du plateau continental. Nous énonçons l'hypothèse que la population de morues du Bonnet flamand s'est maintenue isolée depuis la dernière glaciation.

Received August 16, 1977
Accepted October 7, 1977

Reçu le 16 août 1977
Accepté le 7 octobre 1977

Prior to the middle of the last decade, the most widely used techniques for fish stock discrimination were tag return studies and analysis of geographic variation in morphometric and meristic characters. More recently, electrophoretic analysis of protein polymorphisms has been used to discriminate between stocks of various marine fish species (Ligny 1969). This essentially genetic approach defines a fish stock as a panmictic group of individuals that share a common gene pool and that are more or less isolated during the reproductive period from all other conspecific populations. Such stocks may be distinguished by investigating the geographic distribution of allelomorphic genes over the species range.

In the case of Atlantic cod, *Gadus morhua*, the proteins investigated for polymorphism have included hemoglobin (Sick 1965a, b; Møller 1968; Wilkins 1971), transferrin (Møller 1966; Jamieson 1967, 1975; Jamieson and Otterlind 1971; Jamieson and Jónsson 1971), general serum proteins (Ullrich 1968), and such enzymes as esterase (Nyman 1965), lactate dehydrogenase (Odense et al. 1969), and phosphoglucose isomerase (Dando 1974).

We have analyzed material from one eastern North Atlantic area and a number of western North Atlantic areas for lactate dehydrogenase, NAD-malate dehydrogenase, α-glycerophosphate dehydrogenase, phosphoglucose isomerase, muscle esterase, NADP-isocitrate dehydrogenase, and transferrin (Cross and Payne 1975, 1976). Additionally, we have studied aspartate aminotransferase, phosphoglucomutase, NADP-malate

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Printed in Canada (J4952)
Imprimé au Canada (J4952)

dehydrogenase, 6-phosphogluconate dehydrogenase, mannosephosphate isomerase, and creatine kinase (Cross and Payne unpublished data); but either we were unable to achieve satisfactory electrophoretic resolution of variants for routine phenotyping, or the variants were too rare to be of value for population studies. We found that variation at two loci, *Tf* (transferrin) and *PGI-2* (skeletal muscle phosphoglucose isomerase), was of particular value in distinguishing cod stocks. This paper describes our results on the geographic variation in frequencies of alleles at these two loci for a series of samples taken between 41° and 55°N off eastern North America.

Materials and Methods

Seventeen samples were collected from cod populations covering a wide area of the continental shelf off eastern North America during the period March 1974 to January 1977 (Fig. 1). Offshore samples were collected by bottom trawling. Inshore samples were taken from cod traps or gill nets. Sampling information is given in Table 1. Blood samples were taken from either the heart or the caudal aorta into 3-mL heparinized, evacuated tubes and the plasma was separated by centrifugation at 2000 × *g* for 10 min. Samples of skeletal muscle (caudal peduncle) were also collected. All material was quick-frozen and stored at −20°C. Each specimen was measured and sexed. Otoliths were removed for age determination.

Complete saturation of transferrins with iron was assured by adding to the plasma prior to electrophoresis, one, ½ vol of buffered 10 mM ferric chloride (modification of Payne 1974). Muscle samples were homogenized in equal volumes of cold, 30% dimethyl sulphoxide, 700 mM Tris -HCl, pH 7.5 (Dando 1974); centrifuged for 15 min at 4000 × *g*; and the supernatants used for electrophoresis.

Transferrin variants were resolved by electrophoresis of iron-saturated plasma samples in 5% polyacrylamide gels. The electrode buffer was 18.4 g/L boric acid adjusted to pH 8.6 with NaOH. The concentrated gel-buffer stock solution was 18.2 g/L Tris adjusted to pH 8.6 with citric acid. Gels were prepared by dissolving 15 g Cyanogum 41 (BDH) to 300 mL with 15 mL concentrated gel-buffer stock, 15 mL electrode buffer, 0.3 mL TEMED, and water to the mark; and polymerizing with 3 mL freshly prepared 7% ammonium persulphate in 180 × 180 × 6 mm plastic molds with the usual precautions to exclude atmospheric contact during gelling. PGI isozymes were resolved from skeletal muscle extracts by electrophoresis in 15% starch gels (BDH hydrolyzed starch) with a 135 mM Tris, 45 mM citric acid, pH 7.0, buffer system (Ayala et al. 1972): this buffer was used full strength in the electrode vessels and diluted 1 in 15 for gel preparation.

Samples were applied to the gels on 5-mm squares of Whatman No. 3 filter paper and horizontal electrophoresis was conducted at room temperature with forced-air cooling for 3–5 h at 100–150 V. Transferrins were located by staining the gels for general protein with 0.01% amido black in 5% acetic acid. PGI was located by the method of Scopes (1968): a piece of Whatman No. 1 chromatography paper was cut to appropriate size and soaked with 3 mL of 100 mM Tris -HCl, 10 mM $MgCl_2$ pH 8.0, containing 10 mg fructose-6-phosphate, 9 mg MTT tetrazolium, 5 mg NADP, 1 mg phenazine methosulphate, and 10 U glucose-6-phosphate dehydrogenase (Sigma Type XII); laid on a sliced gel surface and incubated in the dark for approximately 20 min until the bands had developed; peeled off and washed in cold running water; and dried in an oven at 50°C.

Some abbreviations used in this report are the following: PGI, phosphoglucose isomerase (EC 5.3.1.9); *PGI*, a gene locus coding for a PGI subunit; Tf, transferrin; *Tf*, the gene locus coding for this protein; Tris, 2-amino-2-hydroxymethyl-1,3-propanediol; TEMED, *NNN'N'*-tetramethyl-1,2-diaminoethane; BDH, British Drug Houses Ltd.

TABLE 1. Locations of the sampling stations.

Sampling station	Location; ICNAF area[a]	Date of collection
A	Hamilton Inlet Bank; 2J	Nov. 1976
B	Domino (inshore fishery); 2J	Aug. 1976
C	Fox Harbour (inshore fishery); 2J	Aug. 1976
D	N.E. Newfoundland Shelf (N); 2J	Mar. 1974
E	N.E. Newfoundland Shelf (S); 3K	Mar. 1974
F	Northern Grand Bank; 3L	Mar. 1974
G	Northern Grand Bank; 3L	May 1976
H	St. Pierre and Burgeo banks; 3Ps	June 1975
I	St. Pierre and Burgeo banks; 3Ps	May 1976
J	Rose Blanche Bank; 3Pn	Jan. 1976
K	Gulf of St. Lawrence (E); 4R	Jan. 1976
L	Emerald Basin; 4W	Oct. 1975
M	Scotian Shelf; 4W	Oct. 1976
N	Georges Bank; 5Ze	Oct. 1975
O	South Channel; 5Ze	Oct. 1975
P	Flemish Cap; 3M	June 1975
Q	Flemish Cap; 3M	Jan. 1977

[a]International Commission for the Northwest Atlantic Fisheries Statistical Divisions.

Results and Discussion

Transferrin polymorphism (Fig. 2) has been interpreted in terms of six alleles segregating at a single locus, and the transferrin variants are labeled Tf1 to Tf6 in order of decreasing mobilities in the specified electrophoresis system. We presume from comparison of allele frequencies and relative electrophoretic mobilities that our *Tf1* to *Tf6* series corresponds to the *TfA* to *TfE* series of Jamieson (1975), who used a starch-agar gel system (Moller 1966), but as we have been unable to establish a formal correspondence by direct comparison of samples typed in both laboratories we prefer to maintain both taxonomic systems. We have had some difficulties in distinguishing consistently Tf3 and Tf4, which have very similar electrophoretic mobilities, so for the purposes of this paper we are treating *Tf3* and *Tf4* as conallelic.

The *Tf* allele frequency estimates (Table 2) agree quite well with Jamieson's observations and we concur in the decrease of *Tf2* (*TfB*) and increase of *Tf5* (*TfD*) allele frequencies from north to south in the western North Atlantic. However, we are unable to confirm the striking heterozygote deficiencies described

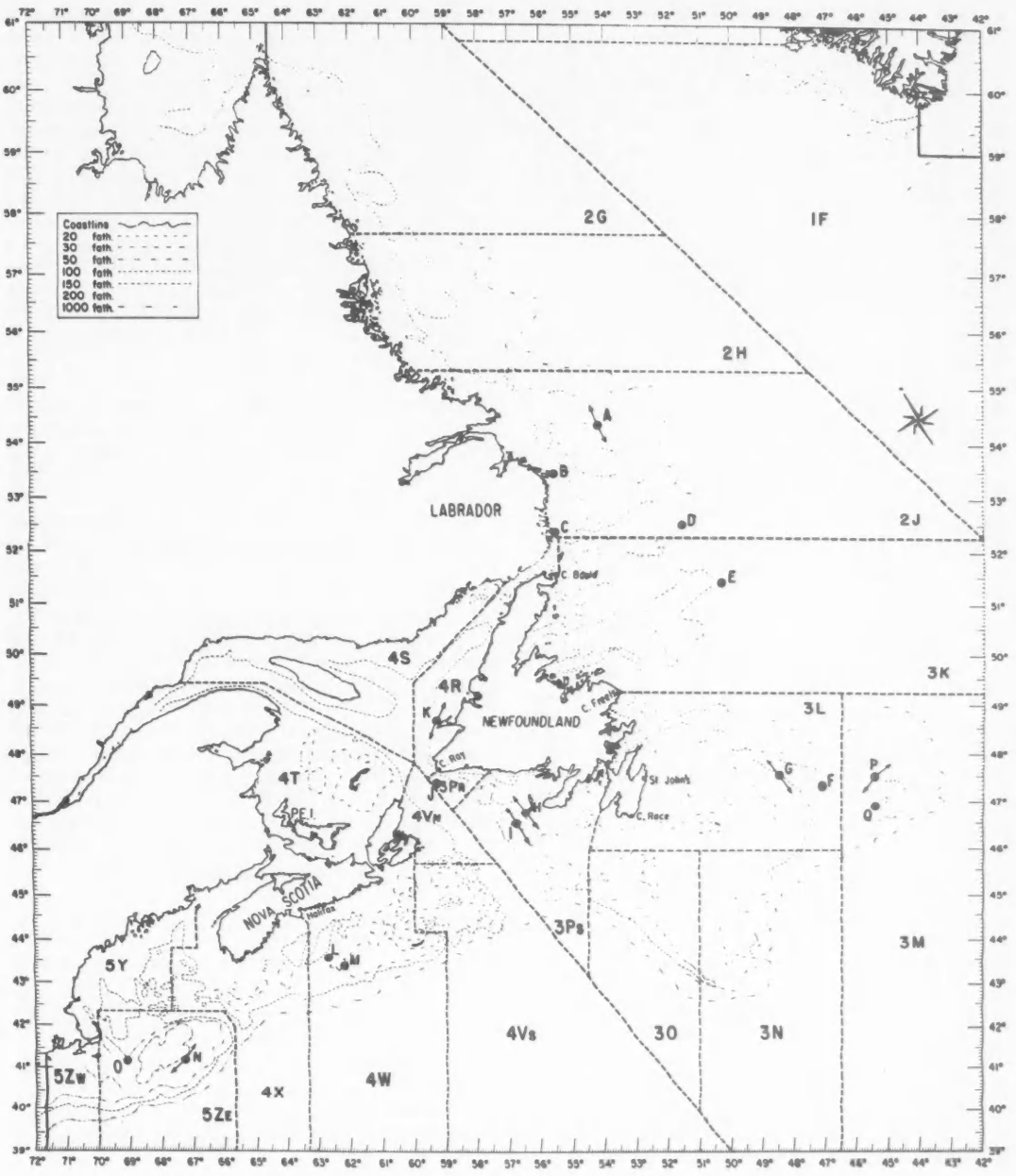

FIG. 1. *Continental shelf off eastern North America showing the sampling locations.*

by Jamieson, which he interpreted as evidence of major genetic divergence at the *Tf* locus for cod inhabiting the various banks off eastern North America. While our results show clear evidence of geographic variation in *Tf* allele frequencies, the observed phenotype frequencies do not differ significantly from Hardy–Weinberg–Castle equilibria expectations for all 17 samples and there is no indication of any discontinuities in allele frequencies of sufficient magnitude to generate heterozygote deficiencies in stratified samples as great

as those described by Jamieson. We were unable to demonstrate an association between transferrin phenotypes and age or sex for our samples.

We have computed a matrix of genetic distances between each of the 17 samples (Table 3) from the *Tf* allele frequency estimates by the method of Rogers (1972) and a phenogram was produced by UPGMA clustering (Sneath and Sokal 1973) of the samples. This phenogram (Fig. 3) clearly demonstrates two major dichotomies (both $P \ll 0.001$) which separate the western North Atlantic cod populations into three main groups: (1) north of the Laurentian Channel, (2) south of the Laurentian Channel, and (3) Flemish Cap.

Within the north Laurentian group, there is also a significant dichotomy ($P < 0.005$) between the samples from off Labrador and northeastern Newfoundland (A–F) and the more southerly samples from St. Pierre and Burgeo banks, Rose Blanche Bank, and the eastern Gulf of St. Lawrence (G–K). However, both clusters include samples from the northern sector of the Grand Bank (F, G); but as these samples were taken in different years and at slightly different seasons the apparent overlap does not necessarily falsify the dichotomy. There may be a zone of transition between the two groups producing a sharp cline similar to that for the frequency of the $Hb\ I^{L}$ hemoglobin allele in the Belt Sea where the Baltic and North Sea cod stocks overlap (Sick 1965a), or the region of contiguity may shift slightly from year to year or seasonally in response to oceanographic conditions. Clearly, further sampling in this area would be of interest.

TABLE 2. Estimated *Tf* allele frequencies. Alleles *Tf3* and *Tf4* are treated as conallelic.

Sampling station	Allele				
	Tf1	*Tf2*	*Tf3/4*	*Tf5*	*Tf6*
A	0.012	0.452	0.397	0.123	0.016
B	0.004	0.440	0.414	0.123	0.019
C	—	0.462	0.444	0.076	0.019
D	0.009	0.469	0.407	0.106	0.009
E	0.019	0.439	0.440	0.098	0.005
F	0.021	0.432	0.453	0.074	0.021
G	0.003	0.416	0.439	0.128	0.014
H	—	0.380	0.486	0.103	0.031
I	0.006	0.388	0.454	0.115	0.038
J	0.007	0.399	0.426	0.149	0.020
K	0.007	0.404	0.463	0.103	0.023
L	—	0.293	0.505	0.172	0.029
M	0.003	0.319	0.436	0.201	0.040
N	—	0.333	0.417	0.213	0.037
O	0.005	0.313	0.491	0.161	0.031
Q	0.120	0.060	0.640	0.165	0.015

Avise and Kitto (1973) have shown that the phosphoglucose isomerase isozymes in a large number of teleost species are the product of dimeric association between the products of two loci: *PGI-1*, which is most active in heart and liver, and *PGI-2*, which predominates in white skeletal muscle. Dando (1974) confirmed the existence of two *PGI* loci in the cod and demonstrated a five-allelic polymorphism at the *PGI-2* locus (Gpi_{β} in his terminology) in a sample of 100 specimens from the English Channel. We have adopted the *PGI* terminology in this paper for we believe that it is preferable to *Gpi* for reasons of more universal applicability in teleost biochemical systematics, typographic convenience, and historical priority.

The *PGI-2* locus is also polymorphic in cod populations off eastern North America and eight distinct phenotypes were resolved from skeletal muscle extracts. As the enzyme is a dimer, the patterns were either single banded or triple banded. The five commonest phenotypes are illustrated in Fig. 4. We interpret the polymorphism in terms of six alleles which we label $PGI\text{-}2^{a,b,c,d,e,g}$ in order of descending electrophoretic

TABLE 3. Matrix of genetic distances between the cod populations sampled based on differences in allele frequencies at the *Tf* locus.

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	Q
A	.															
B	0.016	.														
C	0.048	0.042	.													
D	0.019	0.025	0.036	.												
E	0.037	0.029	0.028	0.033	.											
F	0.055	0.046	0.027	0.049	0.023	.										
G	0.040	0.025	0.049	0.047	0.030	0.043	.									
H	0.083	0.068	0.068	0.086	0.068	0.051	0.047	.								
I	0.063	0.049	0.061	0.070	0.047	0.046	0.030	0.026	.							
J	0.047	0.035	0.070	0.060	0.049	0.062	0.022	0.056	0.035	.						
K	0.060	0.045	0.047	0.062	0.034	0.031	0.027	0.025	0.019	0.042	.					
L	0.141	0.127	0.144	0.151	0.126	0.127	0.104	0.080	0.087	0.095	0.097	.				
M	0.114	0.104	0.135	0.129	0.115	0.122	0.088	0.089	0.079	0.069	0.095	0.057	.			
N	0.108	0.100	0.135	0.124	0.115	0.125	0.087	0.098	0.084	0.067	0.099	0.075	0.019	.		
O	0.122	0.109	0.126	0.132	0.108	0.109	0.086	0.063	0.068	0.077	0.079	0.019	0.049	0.066	.	
Q	0.336	0.325	0.391	0.345	0.315	0.309	0.302	0.269	0.281	0.295	0.288	0.209	0.249	0.266	0.223	.

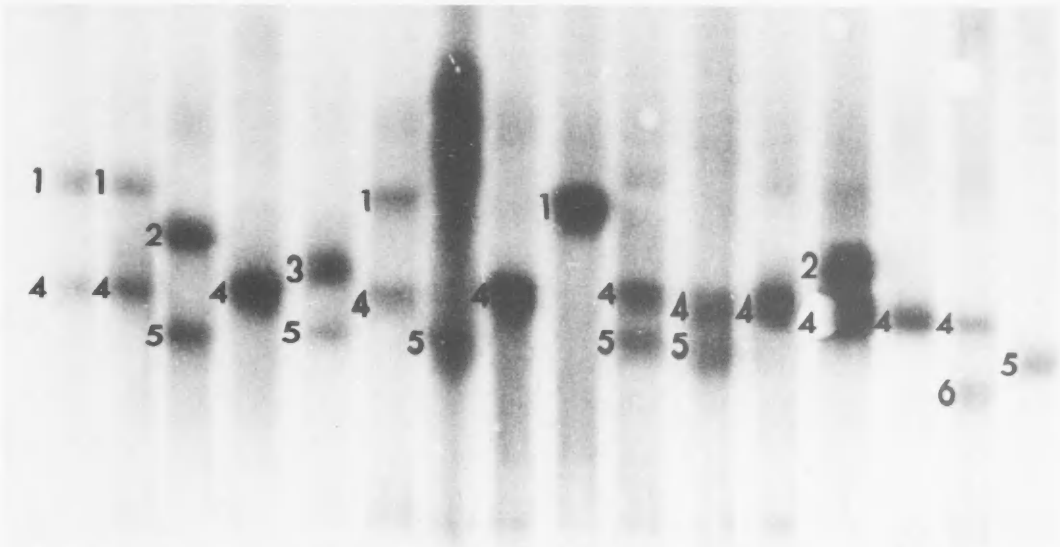

FIG. 2. Portion of a cod serum electropherogram showing some common transferrin phenotypes produced by a hexallelic polymorphism at the *Tf* locus. The different transferrins are labeled from 1 to 6 in order of decreasing electrophoretic mobilities.

TABLE 4. Estimated *PGI-2* allele frequencies.

Sampling station	Allele					
	PGI-2^a	*PGI-2^b*	*PGI-2^c*	*PGI-2^d*	*PGI-2^e*	*PGI-2^g*
A	0	0	0.629	0	0.352	0.019
D	0	0	0.613	0	0.374	0.013
F	0	0	0.686	0	0.296	0.018
H	0	0	0.588	0	0.398	0.014
I	0	0	0.595	0	0.393	0.012
K	0	0	0.614	0	0.379	0.007
L	0	0.006	0.632	0	0.362	0
N	0	0	0.557	0	0.443	0
O	0	0	0.571	0.004	0.408	0.017
P	0.005	0	0.520	0.005	0.460	0.010

mobilities of their homodimeric protein products at pH 7.0. An additional allele, *PGI-2^f*, has only been detected in cod from European waters (Cross and Payne 1976). Our genetic interpretation is supported by a close fit between the observed phenotype frequencies and H–W–C expectations. We were unable to demonstrate variation of phenotype frequencies with age or sex.

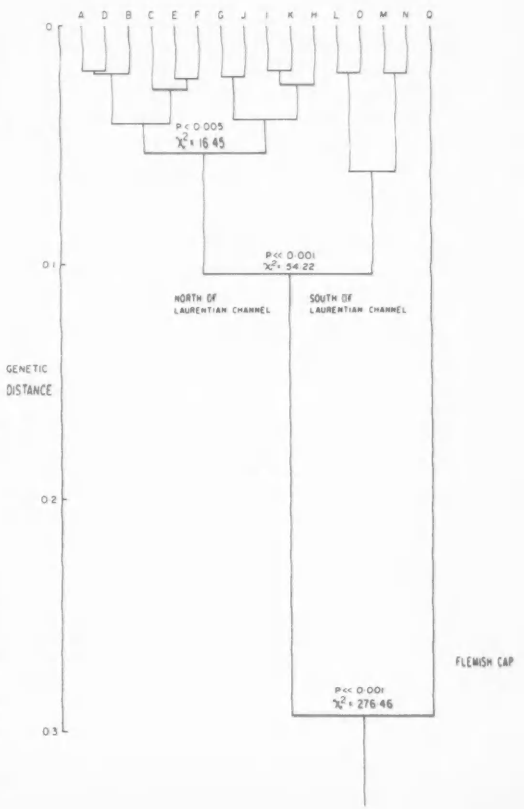

FIG. 3. Phenogram of genetic distances between samples demonstrating the existence of three major stocks of cod off eastern North America: (1) Flemish Cap, (2) south of the Laurentian Channel, and (3) north of the Laurentian Channel. The phenogram also suggests that the north Laurentian stock may be further subdivided into a northern and a southern component. The heterogeneity χ^2 for pooled samples is given at each statistically significant bifurcation.

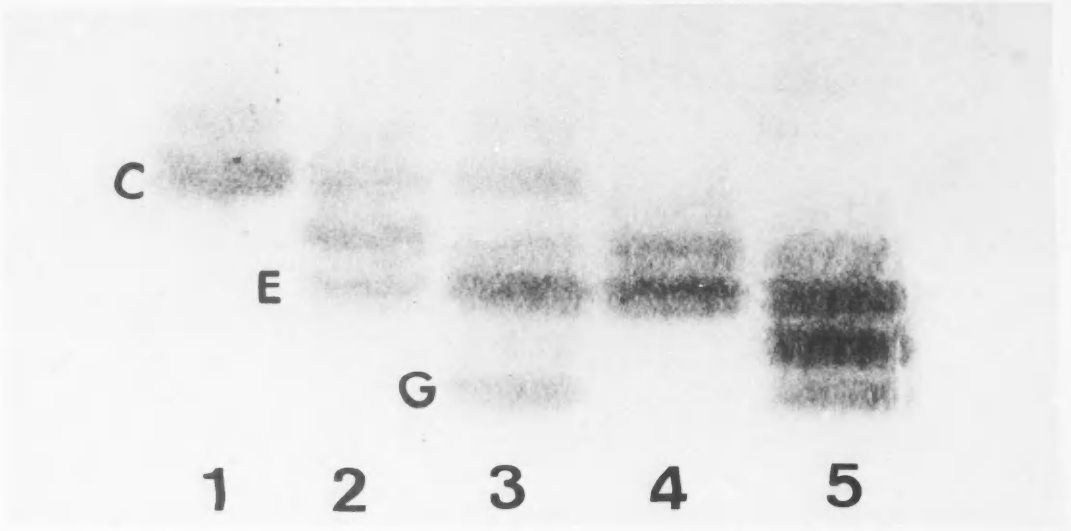

FIG. 4. Electropherogram of cod skeletal muscle extracts stained for phosphoglucose isomerase and showing the five commonest PGI-2 phenotypes. The homodimers are labeled alphabetically in order of decreasing electrophoretic mobilities. The genotypes are interpreted as (1) *PGI-2^c/PGI-2^c*, (2) *PGI-2^c/PGI-2^e*, (3) *PGI-2^c/PGI-2^g*, (4) *PGI-2^e/PGI-2^e*, and (5) *PGI-2^e/PGI-2^g*.

Comparison of allele frequencies from different samples (Table 4) did not show a significant difference in *PGI-2* allele frequencies between the north Laurentian and south Laurentian groups, but we were able to demonstrate significant heterogeneity ($P < 0.005$) between the cod population of Flemish Cap and the adjacent northern Grand Bank. This observation underlines the dramatic genetic divergence that has taken place between Flemish Cap and North American shelf cod populations and we are tempted to speculate that Flemish Cap may have maintained a relict cod population during the last glaciation while the North American shelf cod populations were forced far south of their present range by low sea temperatures and that the two relict groups have not introgressed subsequently. There is no evidence of H–W–C disequilibria on Flemish Cap for either *Tf* or *PGI-2*, so the possibility that these fish are derived by ongoing postzygote selection of larvae drifting across the channel from the shelf population is unlikely. A similar explanation has been proposed (Payne et al 1971; Payne 1974; Child et al. 1976) for the two races of Atlantic salmon in Europe.

Acknowledgments

The authors wish to thank the North Eastern Fisheries Laboratory, Wood's Hole, Mass., USA, for their assistance in our cod sampling programme. One of us (Dr T. F. Cross) was funded by a National Research Council of Canada Postdoctoral Fellowship.

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Utility of Meristic and Morphometric Characters for Identification of Capelin (*Mallotus villosus*) Stocks in Canadian Atlantic Waters[1]

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Multivariate discriminant function analyses were performed on 9 morphometric and 11 meristic characters of capelin (*Mallotus villosus*) samples at Île aux Coudres in the St. Lawrence estuary; Sept Îles, Natashquan, and Grande Rivière in the Gulf of St. Lawrence; Southeast Shoal on the Grand Banks; and Herring Neck, Notre Dame Bay, Newfoundland. Analyses of meristic characters provided no evidence of discrete stocks. Analyses of morphometric characters provided strong statistical separation between areas. A posteriori classification of groups yielded 93.1% correct classification for males and 73.3% for females. Only snout length, eye diameter, head length, and body depth contributed significantly to the separation obtained. These findings suggest that meristic characters offer little potential for stock identification in Canadian Atlantic waters. Morphometric characters may prove useful, but further study, corroborated with life history and migration data, is required to confirm this.

Key words: capelin, *Mallotus villosus*, meristics, morphometrics, stock separation, Gulf of St. Lawrence, Newfoundland, Canadian Atlantic, multivariate analysis

SHARP, J. C., K. W. ABLE, W. C. LEGGETT, AND J. E. CARSCADDEN. 1978. Utility of meristic and morphometric characters for identification of capelin (*Mallotus villosus*) stocks in Canadian Atlantic waters. J. Fish. Res. Board Can. 35: 124–130.

Les auteurs ont procédé à des analyses multivariées de fonctions discriminantes de 9 caractères morphométriques et de 11 caractères méristiques de capelans (*Mallotus villosus*) capturés à l'Île aux Coudres dans l'estuaire du Saint-Laurent; à Sept Îles, à Natashquan et à la Grande Rivière, dans le golfe du Saint-Laurent; au sud-est des hauts-fonds du Grand Banc; à Herring Neck et dans la baie de Notre-Dame, à Terre-Neuve. L'analyse des caractères méristiques n'a pas révélé l'existence de stocks distincts, mais celle des caractères morphométriques a démontré que, du point de vue statistique, il existait une forte démarcation entre les différentes régions. Selon une classification ultérieure des groupes étudiés, 93,1 % des mâles et 73,3 % des femelles ont été classés correctement. La longueur du museau, le diamètre de l'oeil, la longueur de la tête et la hauteur du corps ont été les seuls caractères qui ont réellement contribué à différencier les échantillons. Les résultats semblent montrer qu'il n'est guère possible d'identifier les stocks des eaux canadiennes de l'Atlantique à l'aide des caractères méristiques. À ce chapitre, il se peut que les caractères morphométriques se révèlent utiles, mais il est nécessaire d'entreprendre d'autres études, étoffées par des données sur le cycle évolutif et les migrations, afin de s'en assurer.

Received January 24, 1977
Accepted October 14, 1977

Reçu le 24 janvier 1977
Accepté le 14 octobre 1977

A large foreign fishery for capelin (*Mallotus villosus*) has developed in the Canadian Atlantic since 1972 (Winters 1975). Effective management of this resource requires a knowledge of the occurrence and distribution of distinct spawning stocks. Templeman (1948) and Pitt (1958) attempted to use geographical variation in vertebral numbers to identify capelin stocks in the Newfoundland–Labrador area without success. Payne (1975) also found electrophoretic analysis of liver and skeletal muscle esterases to be ineffective in defining spawning stocks of capelin in Newfoundland waters, although the method did indicate that populations from the Northwest Territories, Newfoundland, and Greenland are genetically distinct (Payne 1975, 1976). In this study we examined, in detail, the possible utility of meristic and morphometric characters in the identification of capelin stocks in Canadian Atlantic waters.

[1]Contribution to the Program of GIROQ (Groupe interuniversitaire de recherches océanographiques du Québec).

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Printed in Canada (J4695)
Imprimé au Canada (J4695)

TABLE 1. Sampling locations and dates, gear and sample sizes.

Site	Latitude	Longitude	Date	Gear	Sample size	
					Male	Female
Île aux Coudres	47°38′70″	70°05′06″	May 1, 1975	Midwater trawl	0	30
Sept Îles	50°07′00″	66°28′30″	May 12, 1975	Midwater trawl	23	30
Natashouan	50°03′45″	61°45′00″	June 10–11, 1975	Midwater trawl	30	30
Grande Rivière	47°58′00″	64°46′30″	June 17, 1975	Cast net	30	30
Southeast Shoal	44°34′00″	50°12′05″	June 29, 1975	Midwater trawl	30	30
Herring Neck	49°30′00″	54°30′00″	July 11, 1975	Cast net	20	30

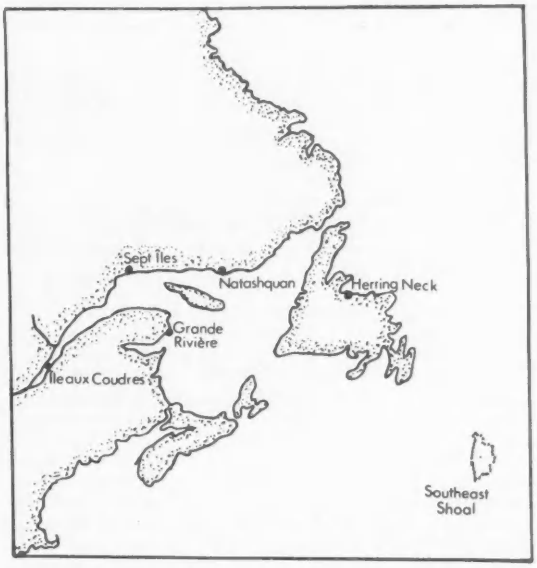

FIG. 1. Sampling locations in the estuary and Gulf of St. Lawrence, Newfoundland, and southeast shoal of the Grand Banks.

Materials and Methods

All specimens used in this study were mature unspent fish collected immediately prior to spawning. Collections were obtained from Île aux Coudres in the estuary of the St. Lawrence River; Sept Îles, Natashquan, and Grande Rivière in the Gulf of St. Lawrence; the Southeast Shoal of the Grand Banks, and from Herring Neck in Notre Dame Bay, Newfoundland (Table 1, Fig. 1). All fish were sealed in plastic bags, frozen immediately, and kept frozen until analysis. All morphometric measurements were made immediately after the fish had thawed.

Nine morphometric and 11 meristic characters (Tables 2, 3) were analyzed. Morphometric measurements and meristic counts were made according to criteria established by Hubbs and Lagler (1967) with the exception of pyloric ceca counts and measurements of pectoral fin length, pelvic to pectoral distance, adipose fin base, snout length and head length which were made according to McAllister (1963). Snout to vent length was measured from the anteriormost portion of the head to the anterior border of the vent. Morphometric measurements were made with dial calipers to an accuracy of 0.1 mm except for snout to vent, snout to dorsal origin, and standard length which were measured with a rule to the nearest millimetre. All morphometric measurements were standardized using the method of Mais (1972) to eliminate the possible influence of different mean fish sizes between samples on intergroup comparisons. Morphometric data were not log-transformed because the length ranges of the fish in the samples was not great (maximum range 135–182 mm) and hence heteroscedasticity about the standardizing regressions was not a significant factor. All meristic counts were performed on cleared and stained specimens (Taylor 1967) except the pyloric ceca counts which were performed before clearing. Males and females were treated separately in all statistical comparisons.

Multivariate discriminant function analyses (Cooley and Lohnes 1971; Bilton 1972; Amos et al. 1963) were used to assess the degree of statistical separation between groups, to evaluate the relative importance of the various meristic and morphometric characters as diagnostic tools, and to develop classification functions for the six groups considered. University of California Biomedical Computer Programs BMD P3D and BMD P7M (Dixon 1975) were used in these analyses. Statistical separation between groups was determined using the Generalized Distance of Mahalanobis (Mahalanobis D^2). This function provides a measure of the distance between groups which is based on a combination of characters, each considered only to the extent that it is uncorrelated with characters previously employed in the analysis (Mais 1972; Blackith and Reyment 1971). Mahalanobis D^2 was converted to percent overlap, a measure of the overlapping area under two normal curves, which indicates the maximum extent to which two groups share identical characteristics (Mais 1972; Royce 1964).

Results

Multivariate analyses of meristic characters yielded high overlap values between samples (Table 2). A posteriori classification to groups using classification functions derived from meristic data yielded poor overall success with only 18.9% of females and 27.1% of males being correctly classified as to origin of sample.

Multivariate analysis of morphometric values indicated strong significant differences between groups. Percent overlap values for between group comparisons were consistently lower than those obtained with meristic data (Table 3). The degree of separation be-

TABLE 2. Corrected Mahalanobis D^2 and percent overlap comparisons of female (below diagonal) and male (above diagonal) meristic characters. (left pectoral fin rays; dorsal fin rays; anal fin rays; pyloric ceca; brachiostegal rays; gill rakers on upper limb; gill rakers on lower limb; dorsal secondary caudal rays; ventral secondary rays; caudal vertebrae; predaudal vertebrae).

	Grande Rivière		Natashquan		Sept Îles		Île aux Coudres		Herring Neck		Southeast Shoal		
	D^2	% overlap	D^2	% overlap	D^2	% overlap	D^2	% overlap	D^2	% overlap	D^2	% overlap	
Grande Rivière			2.1648	52.22	1.3563	56.20	—	—	3.1950	37.34	3.0711	38.44	Grande Rivière
Natashquan	2.3365	44.77			1.0339	61.00	—	—	2.6184	58.92	3.4135	35.76	Natashquan
Sept Îles	2.3646	44.12	0.9465	62.42			—	—	1.1602	58.92	4.6697	28.02	Sept Îles
Île aux Coudres	1.4293	54.84	0.7299	66.72	1.2870	56.86			—	—	—	—	Île aux Coudres
Herring Neck	8.0798	15.56	4.8639	27.14	3.9197	32.22	6.3273	20.76			6.3665	20.76	Herring Neck
Southeast Shoal	2.7967	40.08	2.8102	40.01	2.2562	45.32	3.1850	37.34	1.3562	56.70			Southeast Shoal

TABLE 3. Corrected Mahalanobis D^2 and percent overlap comparisons of standardized female (below diagonal) and male (above diagonal) morphometric characters (snout to vent length; snout to dorsal origin; body depth; snout length; adipose fin base; eye diameter; pelvic to pectoral length; pectoral fin length; head length).

	Grande Rivière		Natashquan		Sept Îles		Île aux Coudres		Herring Neck		Southeast Shoal		
	D^2	% overlap	D^2	% overlap	D^2	% overlap	D^2	% overlap	D^2	% overlap	D^2	% overlap	
Grande Rivière			28.1107	<1%	71.9552	<1%	—	—	11.3579	4.34	24.3208	1.24	Grande Rivière
Natashquan	17.4355	1.02			138.5398	<1%	—	—	42.7971	<1%	68.5689	<1%	Natashquan
Sept Îles	5.7388	14.42	10.2976	10.42			—	—	57.0203	<1%	11.3836	9.10	Sept Îles
Île aux Coudres	2.7205	37.34	17.8644	2.32	5.4293	23.40			—	—	—	—	Île aux Coudres
Herring Neck	13.0091	5.62	2.1551	43.54	7.8060	15.56	16.7258	3.08			23.3693	1.32	Herring Neck
Southeast Shoal	6.9322	14.42	8.8279	10.96	10.2653	10.96	6.8176	13.62	7.2770	17.38			Southeast Shoal

tween groups for males was very high with 6 of 10 comparisons yielding overlap values of less than 1%.

Stepwise discriminant function analysis revealed that only four of the nine morphometric characters investigated contributed significantly to the multivariate discrimination between groups of males and females. These were, in order of importance, snout length, eye diameter, head length, and body depth for females and eye diameter, snout length, head length, and body depth for males (Table 4). In these measures, as contrasted with snout to vent length, snout to dorsal origin, and pelvic top pectoral distance, self correlation with the standardizing variable (standard length) was small. In self correlation, slopes are biased toward 1.0 and intercepts toward 0.0. This bias tends to obscure real differences between populations and may have been a factor in the elimination of the latter measures from the discriminant function. Some self correlation is inevitable in all such standardizations and hence the separations achieved here are minimum estimates. A posteriori classification to groups using classification functions derived from the morphometric characters (Table 5) yielded strong separation with 93.1% of males and 73.3% of females being correctly classified as to sample of origin (Table 6). The significance of intergroup differences (D^2) based on these characters exceed the $P = 0.05$ level in every comparison for males and females (Table 7).

TABLE 4. F values for test of significance of contribution of various morphometric characters to multivariate discrimination between groups.** $P < 0.01$; other F values were not significant at 5% level.

Variable	F	Variable	F
	♂ ($df = 4, 122$)		
Eye diameter	214.52**	Snout–dorsal origin	3.46
Snout length	97.63**	Adipose fin base	2.04
Head length	27.17**	Pelvic–pectoral distance	1.18
Body depth	16.68**	Pectoral fin length	0.98
Snout–vent length	3.55		
	♀ ($df = 5, 171$)		
Snout length	56.25**	Snout–dorsal origin	2.52
Eye diameter	23.56**	Adipose fin base	2.04
Head length	13.11**	Pelvic–pectoral distance	1.18
Body depth	8.81**	Pectoral fin length	0.98
Snout–vent length	1.31		

Plots of the first two canonical variables for males and females, illustrating the degree of separation between groups on two planes, are presented in Fig. 2 and 3, respectively.

TABLE 5. Classification functions for male and female capelin from six geographical areas (based on standardized morphometric values).

Variable	Southeast Shoal	Herring Neck	Grande Rivière	Natashquan	Sept Îles
			♂		
Snout length	10.96745	10.37540	9.27145	5.45811	5.30035
Eye diameter	25.98134	15.81290	19.17099	15.65727	28.52165
Head length	1.28924	1.45384	0.79073	2.05803	2.24040
Body depth	0.73970	0.99375	2.00313	1.39401	1.25663
Constant	−207.43517	−147.51077	−154.13966	−119.65422	−202.28496

Variable	Southeast Shoal	Herring Neck	Grande Rivière	Natashquan	Sept Îles	Île aux Coudres
			♀			
Snout length	12.20089	11.19069	16.12691	10.35209	14.08489	15.55724
Eye diameter	14.07463	9.45114	11.31585	10.18685	8.61440	12.34431
Head length	16.90643	16.97420	15.06923	17.21227	16.71426	15.88995
Body depth	4.20101	4.72462	4.86902	4.02578	4.33983	4.42892
Constant	−447.47095	−425.65430	−400.17871	−410.70581	−439.62793	−453.76172

TABLE 6. Percent correct a posteriori classification to groups based on morphometric classification functions.

	Males No. cases classified into group					
Group	Southeast Shoal	Herring Neck	Grande Rivière	Natashquan	Sept Îles	Percent correct
Natashquan	0	0	0	30	0	100.0
Grande Rivière	0	2	28	0	0	93.3
Sept Îles	2	0	0	0	21	91.3
Southeast Shoal	27	0	2	0	1	90.0
Herring Neck	0	15	2	0	0	88.2
Total	29	17	32	30	22	93.1

	Females No. cases classified into group						
Group	Southeast Shoal	Herring Neck	Grande Rivière	Natashquan	Sept Îles	Île aux Coudres	Percent correct
Natashquan	2	2	0	25	1	0	83.3
Grand Rivière	0	0	24	0	0	6	80.0
Sept Îles	1	5	2	1	19	2	63.3
Southeast Shoal	24	3	0	1	1	1	80.0
Herring Neck	2	19	1	7	1	0	63.3
Île aux Coudres	1	0	3	0	5	21	70.0
Total	30	29	30	34	27	30	73.3

Discussion

Multivariate analysis of meristic characters provided no evidence of separate stocks of capelin, yet the lowest overlap values consistently occurred in comparisons between the Atlantic and Gulf samples while the highest overlap values occurred in comparisons between Gulf samples. In spite of the much larger number of characters investigated, this finding did not differ from that of earlier workers who studied differences in vertebral number as a possible means of stock separation (Templeman 1948, 1968; Pitt 1958). This finding indicates that analysis of meristic characters offers little promise as a diagnostic tool in the classification of separate stocks of capelin in the Canadian Atlantic area.

In sharp contrast, multivariate analysis of morphometric characters provided strong separation between the six groups compared. Only two comparisons (Île aux Coudres vs. Grande Rivière and Herring Neck vs. Natashquan) yielded overlap values in excess of 25%,

TABLE 7. F values for significance of D^2 between groups (based on standardized measures of snout length, eye diameter, head length, and body depth). $*P < 0.05$, $**P < 0.01$.

	Southeast Shoal	Herring Neck	Grande Rivière	Natashquan
Herring Neck	79.76**			
Grande Rivière	72.07**	21.46**		
Natashquan	196.58**	37.54**	62.71**	
Sept Îles	62.36**	126.56**	105.38**	145.16**

	Southeast Shoal	Herring Neck	Grande Rivière	Natashquan	Sept Îles
Herring Neck	30.49**				
Grande Rivière	38.82**	52.57**			
Natashquan	23.73**	8.40*	69.73**		
Sept Îles	27.31**	16.01**	23.97**	23.26**	
Île aux Coudres	19.56**	49.97**	6.63*	56.19**	19.55**

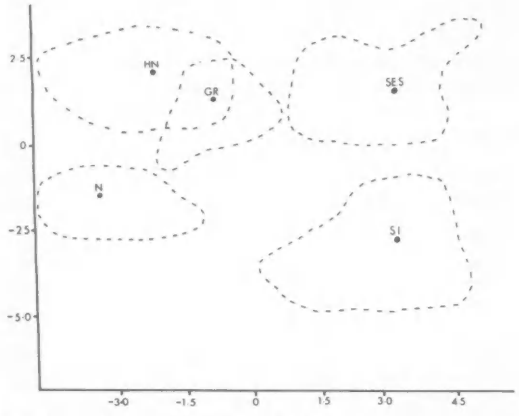

FIG. 2. Relative separation of six male capelin samples based on plots of first (x axis) and second (y axis) canonical variables of four morphometric characters. Closed circles = group means, contours enclose all cases in group. IC = Île aux Coudres, Gr = Grande Rivière, SI = Sept Îles, SES = Southeast Shoal, HN = Herring Neck, N = Natashquan.

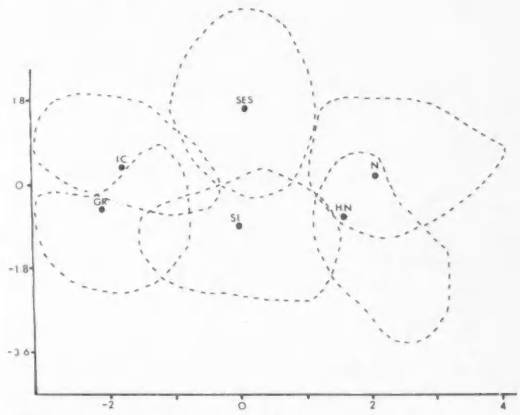

FIG. 3. Relative separation of six female capelin samples based on plots of first (x axis) and second (y axis) canonical variables of four morphometric characters. See Fig. 2 for explanation of symbols.

the level cited by Mais (1972) as providing clear indication of separate stocks of sardines, the average for the remainder being approximately 7%. A posteriori classification of the 310 capelin in the total sample to the six groups, based on classification functions derived from the analysis, was also quite precise. Separation was greater for males than for females and this was reflected in the success of a posteriori classification, the success rate for females being approximately 20% less than that for males.

The results of these analyses suggest that morphometric characters may be useful in separating and classifying stocks of capelin. The statistical separation obtained between the Southeast Shoal and Herring Neck samples is consistent with the conclusion of Campbell and Winters (1973), based on an analysis of seasonal distribution and probable migration patterns, that the Southeast Shoal and Notre Dame Bay populations represent separate stocks. The pronounced north–south cline in mean age of capelin spawning in Canadian Atlantic waters (Winters 1974; Winters and Campbell 1974) and the extension of this trend into the estuary and Gulf of the St. Lawrence (Bailey et al. 1977) may also be a reflection of the presence of separate stocks. However, caution must be exercised in interpreting the results of the analysis of morphometric characteristics provided above since Mayre et al. (1953), Royce (1953, 1957), and Ahlstrom (1957) have shown that statistically significant morphological differences may occur even between closely related populations. The statistical separation achieved between different populations sampled in the estuary and Gulf, which experience larval intermixing as a result of prevailing currents

(Jacquaz et al. 1977), possibly resulted from discrete sampling of a cline in these characters throughout the region. However, Kilambi (1965), Kilambi et al. (1967), and Hart and McHugh (1944), using serological, morphological, meristic, and parasitic indicators, have documented the existence of discrete stocks in two other marine Osmerids, *Hypomosus pretiosus* and *Thaleichthyes pacificus*, having life histories similar to capelin. In one case (Kilambi et al. 1967) the two stocks were separated by distances smaller than those occurring between the Gulf capelin stocks studied here.

A further verification of the validity of this technique is needed. This will require a more intensive sampling of capelin from the estuary, Gulf, Newfoundland, and Labrador waters. In view of the possibility of finding significant differences between closely related populations, the results of these studies should be corroborated by parallel investigations of the population dynamics and migratory patterns of the populations spawning in these areas.

Acknowledgments

Financial and logistical support for this investigation were provided by Groupe interuniversitaire de recherches océanographiques du Québec (GIROQ); Canada Department of the Environment, Fisheries and Marine Services, Quebec; Ministère de l'Industrie et du Commerce, Québec, Direction des Pêches Maritimes; and the National Research Council of Canada (Grant No. A6513). B. Riddell assisted with the statistical analysis. We are also indebted to the reviewers for their constructive criticism.

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Reproductive Isolation Between Pumpkinseed (*Lepomis gibbosus*) and Longear Sunfish (*L. megalotis*) (Centrarchidae) in the Thames River, Southwestern Ontario

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KEENLEYSIDE, M. H. A. 1978. Reproductive isolation between pumpkinseed (*Lepomis gibbosus*) and longear sunfish (*L. megalotis*) (Centrarchidae) in the Thames River, southwestern Ontario. J. Fish. Res. Board Can. 35: 131–135.

In the middle branch of the Thames River, southwestern Ontario, nest construction and occupation by male pumpkinseeds (*Lepomis gibbosus*) began earlier and reached a peak earlier in the season than nesting by male longear sunfish (*L. megalotis*). Nevertheless the two species' breeding seasons broadly overlapped. All pumpkinseed nests occurred in backwater areas, where water flow is minimal, the substrate is a deep layer of detritus and mud, and aquatic plant growth is heavy. Some longear nests were in the backwaters, but most were in the main river, where water flow is variable, the substrate mainly sand and gravel, and vegetation sparse. Females of both species are nonterritorial and were found throughout the study area. Hybrid sunfish are present in the area and opportunities for interspecific breeding would seem to be greatest in backwaters, where males of both species may occupy nests close to each other. However, the only observed interbreeding was between female pumpkinseeds and male longears in the main river. Behavioral barriers to interbreeding occasionally break down under the turbulent social conditions associated with breeding in large longear colonies.

Key words: sunfish, reproductive isolation, longear, pumpkinseed, habitat, season, behavior

KEENLEYSIDE, M. H. A. 1978. Reproductive isolation between pumpkinseed (*Lepomis gibbosus*) and longear sunfish (*L. megalotis*) (Centrarchidae) in the Thames River, southwestern Ontario. J. Fish. Res. Board Can. 35: 131–135.

Dans le bras central de la rivière Thames, dans le sud-ouest de l'Ontario, la construction et l'occupation des nids par les crapets-soleils mâles (*Lepomis gibbosus*) ont débuté et atteint leur maximum plus tôt dans la saison que chez les crapets à longues oreilles mâles (*L. megalotis*). Néanmoins, les deux saisons de frai se sont grandement chevauchées. Tous les nids des crapets-soleils étaient situés dans des zones où: le courant était très faible, le fond, constitué d'une couche importante de débris et de boue, et la végétation aquatique, dense. Bien qu'il y eût aussi quelques nids de crapets à longues oreilles, la plupart d'entre eux se trouvaient dans le bras principal où le courant était variable, le fond, surtout sableux et graveleux, et la végétation, clairsemée. Les femelles des deux espèces n'occupaient pas de territoires bien définis et ont été aperçues dans toute la zone étudiée. Des crapets hybrides étaient aussi présents. Les conditions ont semblé plus favorables à des croisements interspécifiques dans les zones où le courant était minimal en raison de la proximité des nids occupés par les mâles des deux espèces. Toutefois, les seuls cas observés l'ont été dans le bras principal, entre des crapets-soleils femelles et des crapets à longues oreilles mâles. Le frai dans les sociétés nombreuses de crapets à longues oreilles crée des conditions sociales instables qui entraînent occasionnellement la disparition des comportements opposés au croisement interspécifique.

Received July 11, 1977
Accepted September 29, 1977

Reçu le 11 juillet 1977
Accepté le 29 septembre 1977

HYBRIDIZATION is common among sunfishes (Centrarchidae), especially in the genus *Lepomis* (Breder 1936; Hubbs 1955; Slastenenko 1957; Childers 1967; Colgan et al. 1976). Commonly cited causes are crowding of breeding areas because of competition for limited suitable spawning sites (Bailey and Lagler 1938; Birdsong and Yerger 1967) and inability of some fish to find conspecific mates because of great disparity in numbers of the different species (Hubbs 1955). The possi-

Printed in Canada (J4916)
Imprimé au Canada (J4916)

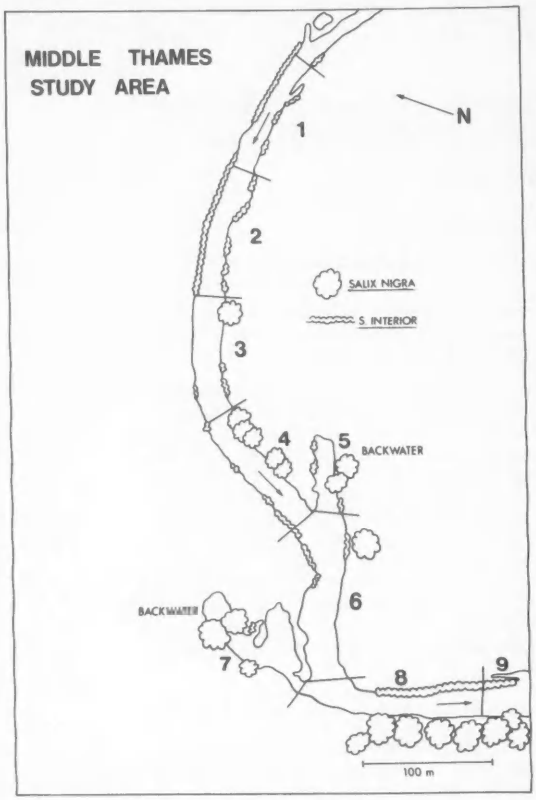

FIG. 1. Study area showing the nine numbered subdivisions of which 5 and 7 are the backwaters, and locations of two species of willows (*Salix*).

bility of interbreeding is enhanced in many areas by the similarity in breeding seasons and habitats of some species (Breder 1936; Clark and Keenleyside 1967; Smith 1970; Scott and Crossman 1973).

A population consisting of longear sunfish (*Lepomis megalotis*), pumpkinseeds (*L. gibbosus*), and their hybrid has been under study for several years in the Thames River system of southwestern Ontario (Keenleyside 1971, 1972; Steele and Keenleyside 1971). Despite the presence of hybrids, the large majority of *Lepomis* in the study area are clearly identifiable as longears or pumpkinseeds. Therefore, introgression and swamping of the two parental gene pools do not seem to be occurring. A major goal of this work has been to explain the continued formation of the hybrids. This paper presents data on seasonal, habitat, and ethological isolating mechanisms collected during two breeding seasons in the Thames River study area.

Study area — The study area, a 700 m-long stretch of the middle branch of the Thames River, about 24 km east of London, Ont., contains two distinct habitat types (Fig. 1). One is the main river, where water flow is usually slow to moderate, substrate is sand and gravel, overlain with a thin layer of silt, and water depths in the summer low-water period range from a few centimetres along the edges to about 1 m in the few pools. During occasional summer floods following heavy rains the maximum depth rises to about 1.5 m and the water is fast and turbid. The second habitat consists of two backwaters with virtually no water flow, where the substrate consists of deep, soft layers of mud and detritus and the water is 1 m deep or less. To study sunfish distribution within these two habitat types, the study area was divided into nine subdivisions (Fig. 1). Seven of these were in the main river; Areas 5 and 7 were the two backwaters.

Materials and methods — Sunfish nesting activity was monitored throughout the breeding seasons of 1968 and 1969 by the same observer. Each day, when weather and water conditions permitted sighting of nests, he walked the entire length of the study area, up one shoreline and down the other. The numbers of nests occupied by male longears and male pumpkinseeds in each of the nine subdivisions were recorded. Sunfish nests are bowl- or saucer-shaped depressions excavated in the substrate by vigorous tail-beating movements of the male (Miller 1963). A nest was scored as occupied if a male sunfish was resident, i.e. maintained position in the nest and aggressively kept other sunfish from entering it.

Female sunfish numbers were determined by a regular seining program, rather than by observation, because females are nonterritorial and spend much time in pools and under overhanging willows. Twice a month, during May, June, and July of both years, each subdivision was fished once in a standard manner with a 20 × 2 m seine. All sunfish captured were identified to species, measured, and immediately released in the area of capture. Adjacent areas were not seined on the same day or on 2 consecutive days; this reduced the possibility of recapturing fish that might have temporarily moved away from their capture location after release.

A total of 24 h in each year of the study was spent by an observer sitting quietly on the river bank, watching the fish during spawning. Much of this time was spent observing the backwater areas, where males of both species occasionally nested close to each other.

Results — (a) Seasonal isolation — In 1968 and 1969 some male pumpkinseeds established nests earlier in the season than male longears did (Fig. 2 and 3). Also the highest peak in numbers of occupied nests was reached sooner by pumpkinseeds. Despite these differences there was a period of 4–6 wk each year when many males of both species actively maintained nests in the study area.

Figures 2 and 3 illustrate another interspecific difference in nesting behavior. The total number of nests occupied by longear males declined suddenly and then rapidly rose to a new peak, part way through the breeding season each year. A similar fall and rise in nest occupancy did not occur at the same time with pumpkinseeds. These rapid changes in numbers of occupied longear nests were caused by a marked increase in water level, turbidity, and speed in the river following several days of heavy rainfall. Sunfish nests in the river

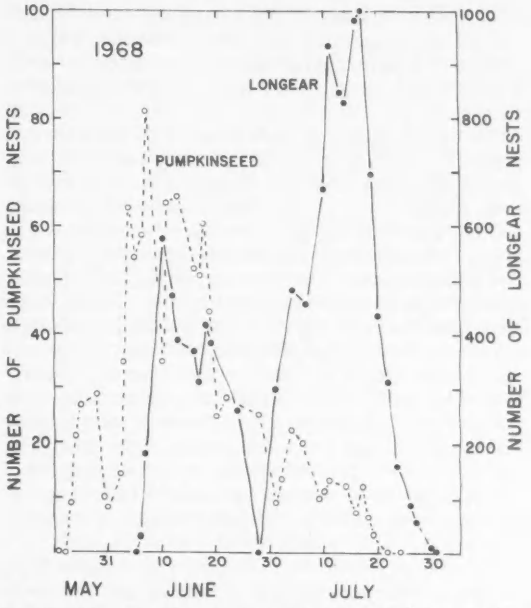

FIG. 2. Number of nests in study area occupied by males of the two species during 1968 breeding season.

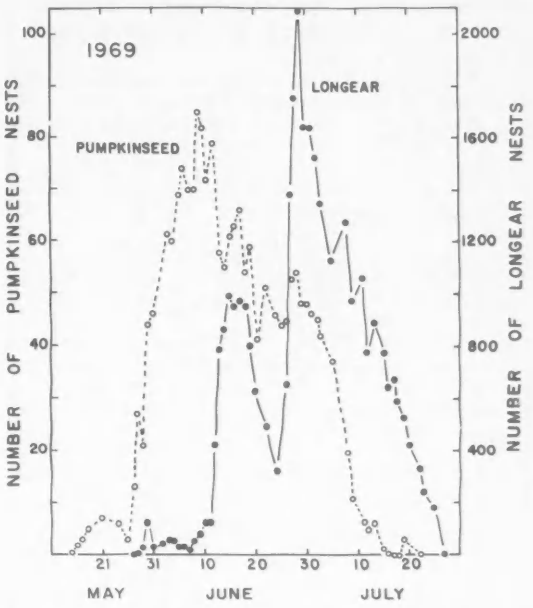

FIG. 3. Number of nests in study area occupied by males of the two species during 1969 breeding season.

are vulnerable to such flooding, since they can be quickly filled in by shifting substrate. Longear males desert their nests under these conditions, although they may build new nests in the same area when the flood waters subside. Pumpkinseed nests were found only in the backwaters (see below), where they are not subject to the same scouring and silting conditions, even when the main river is in full flood.

(b) Habitat isolation — Pumpkinseed males nested only in the backwaters, while most, but not all, longear males nested in the river in both years (Fig. 4 and 5). Occupied longear nests were only found in the backwaters within the period when pumpkinseeds were also nesting there. Thus a mature female sunfish entering a backwater might have encountered nest-guarding males of both species, whereas in the river she would only have encountered nesting longears. Partial breeding habitat isolation therefore existed in the study area. However, the two backwaters contained dense stands of aquatic plants that probably restricted the fishes' visual field. Thus the precise locations of backwater nests would influence the probability of interspecific encounters.

Some data are available on microdistribution of backwater nests. Figure 6 shows the locations of nests occupied by males of both species in Area 5 on June 7, 1968. The proximity of some pumpkinseed and longear nests is obvious. Spawning occurred on June 7 in the three longear colonies and in three pumpkinseed nests, as indicated on Fig. 6.

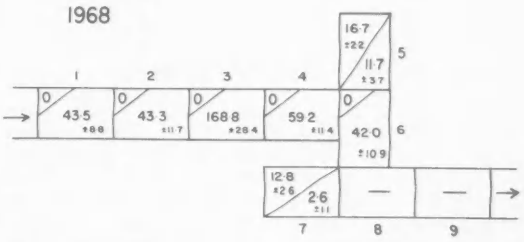

FIG. 4. Simplified outline of study area showing mean number of nests (±1 SE) per day in each subdivision during 1968 breeding season. Value in upper left of each section, pumpkinseed nests; value in lower right, longear nests. No data collected in Areas 8 and 9.

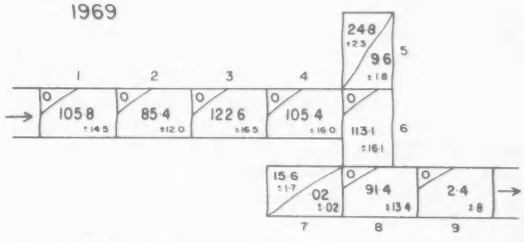

FIG. 5. Mean number of nests (±1 SE) per day in all nine subdivisions during 1969 breeding season. Details as for Fig. 4.

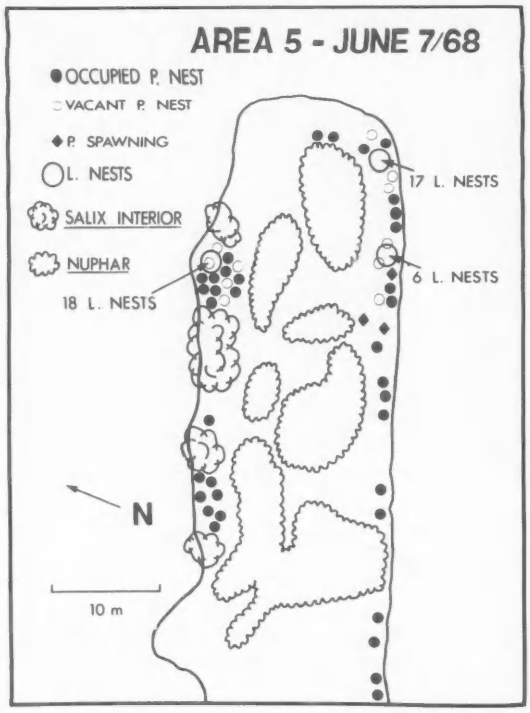

FIG. 6. Locations of all pumpkinseed and longear nests in Area 5 on June 7, 1968. Some *Salix* symbols have been removed for clarity. P., pumpkinseed; L., longear.

These data provide information on spawning sites for the two species, but say nothing about distribution of female sunfish during the breeding season. The seining program showed that adult females of both species were common in the river and the backwater habitats throughout the breeding season in both years. Disparity in numbers between the two types was greatest in the river, where longear females were the most common.

(c) Ethological isolation — From June 3 to 9, 1968 the pumpkinseed and longear nests of Area 5 (Fig. 6) were closely observed for several hours each day. The colony of 18 longear nests was of particular interest. On June 3 five pumpkinseed nests were present at that location; each was defended by an active, territorial male guarding eggs or fry. Longear males were seen there for the first time on June 4, when they showed low-intensity territorial behavior. By June 7 more longear males were present, and many were actively defending nests within 1 m of the pumpkinseed nests. Female longears were also in the vicinity, and several longear spawnings were seen that day. Two of the pumpkinseed nests had been deserted, while three remained occupied. Part of the area had, in effect, been "taken over" by longear sunfish. Female pumpkinseeds were not seen after June 3 in or near this cluster of nests, even though pumpkinseeds spawned elsewhere in Area 5 on June 7 (Fig. 6). Male pumpkinseeds were never seen to court female longears, but were highly aggressive towards them.

On several occasions during early July 1969, female pumpkinseeds were observed spawning with male longears in the main river. All of these cases occurred in large longear colonies when there was much spawning activity, including intrusion and disruption of spawning longear pairs (Keenleyside 1972) in the same colonies. An interesting feature of these spawnings was the continuous high level of aggression by the resident male. He frequently broke off the circling behavior, which is typical of breeding *Lepomis* species (Breder and Rosen 1966), and vigorously attacked the female pumpkinseed. The latter usually resisted the attacks, stayed in the nest, and continued circling. The male soon stopped his aggression and resumed circling with her. The pumpkinseed females were seen to release eggs, and as far as could be determined, these interspecific encounters led to deposition of fertilized eggs in the longear nests.

The seining program also provided evidence that interactions between female pumpkinseeds and male longears may be common in the river habitat. Ripe female pumpkinseeds were often captured on the fringes of large longear colonies, particularly when spawning activity in these colonies was high.

Mean lengths of adult *Lepomis* collected by seining in May, June, and July 1969 showed that pumpkinseeds are larger than longears, and among longears, males are larger than females ($P < 0.001$, t-test). Mean total lengths in centimetres ($n = 200$ for each sample): *L. gibbosus* males, 13.44 (SE, 0.163), females, 13.19 (0.177); *L. megalotis* males, 9.02 (0.074), females, 7.76 (0.058).

Discussion — Seasonal and habitat barriers to reproduction between longear sunfish and pumpkinseeds are incomplete in the Middle Thames study area. During a 3- to 4-wk period in June and July many males of both species actively maintain and defend nests. In the backwater sections, both types of males occasionally occupy nests within a few centimetres of each other. This proximity between nesting fish does not occur in the main river, where only male longears maintain nests. On the other hand, the females are mobile and nonterritorial and are found throughout the breeding season in backwaters and main river. Thus the important question is, what is the probability of a prolonged encounter between a nest-occupying male of one species and a mature female of the other species? Several types of evidence bear on this question.

First, no interspecific breeding was seen in the backwaters, despite many hours spent observing fish in areas where nests of both species were close together. The marked aggressiveness of nesting male pumpkinseeds and the absence of female pumpkinseeds from

these crowded backwater nesting situations seemed to preclude interspecific breeding.

Second, breeding between male longears and female pumpkinseeds was seen several times in longear river colonies. The frequent attacks on the female by the resident male and the female's continued resistance to these attacks suggest that these encounters were primarily caused by persistent efforts of the female pumpkinseed to spawn with the longear male, rather than by active courtship by the male. The number of occupied pumpkinseed nests was rapidly declining when these interspecific spawnings were seen (early July 1969, see Fig. 3), and ripe female pumpkinseeds may have been induced to approach nesting longear males because they were unable to locate suitable conspecific males.

Earlier laboratory experiments on mate selection support the idea that the male longear–female pumpkinseed cross is the most likely source of hybrids in the area. One study showed that males of each species directed more courtship acts towards conspecific than heterospecific females and that this preferential courtship was more pronounced among male pumpkinseeds than male longears (Keenleyside 1967). In another study females of both species responded more strongly towards conspecific than heterospecific males, and this differential response was most pronounced among longear females (Steele and Keenleyside 1971). As a result of these two studies, Steele and Keenleyside (1971) proposed a hybridization model by which interspecific breeding in the study area might be expected to be most common between male longears and female pumpkinseeds. The field observations described in the present paper support this hypothesis.

Further support stems from the size data (p. 134). Nesting males of both species are highly aggressive, and since male pumpkinseeds are much larger than female longears, whereas male longears are smaller than female pumpkinseeds, a female longear may well be more easily inhibited from approaching a nest guarded by a male of the other species than is a female pumpkinseed. No experimental data are available to test this suggestion.

The possibility remains that hybridization occurs accidentally, by sperm drifting from one spawning location to fertilize eggs in another. Since pumpkinseed nests are not maintained in the river habitat, accidental cross-fertilization will not occur there. In the backwaters, even if the two species spawn simultaneously, there is so little water flow that sperm is not likely to be carried far from the point of release. The movements of the two fish during spawning could result in some sperm being washed out of the nest, but no direct evidence is available on the probability of sperm drifting from one nest to another in relatively still-water conditions. In addition, since the sperm of several *Lepomis* species is known to have a functional life span of about 1 min (Childers 1967), sperm drift as a cause of hybridization seems unlikely in the backwater habitat.

Acknowledgments — Much of the data presented here was collected by J. Cresswell and a number of student assistants. For other contributions to the work I wish to thank K. Bruen, J. Harwood, R. Steele, A. Blauboer, and D. May. I am grateful to B. Bietz, M. Evered, and C. Prince for commenting on a draft of the paper. The research was supported by the National Research Council of Canada.

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Estimating the Diet of a Sluggish Predator from Field Observations

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PETERSON, C. H., AND B. P. BRADLEY. 1978. Estimating the diet of a sluggish predator from field observations. J. Fish. Res. Board Can. 35: 136–141.

Predation often occurs slowly, so that observing a sufficiently large sample of entire predation episodes represents an inconvenient means of quantitatively estimating the predator's diet. Fortunately, related data are usually available specifying the proportions of predators found feeding on each prey type. We demonstrate how dividing the observed proportion of all predators found feeding on a given prey type by the average contact time required to consume a single item of that prey type produces an estimate of the relative frequency of the prey in the predator's diet. The analogous procedure, when based upon residence times in the gut, serves to derive a quantitative estimate of diet from numerical data on gut contents.

Key words: dietary analysis, feeding frequencies, stomach contents, predator–prey contact times

PETERSON, C. H., AND B. P. BRADLEY. 1978. Estimating the diet of a sluggish predator from field observations. J. Fish. Res. Board Can. 35: 136–141.

La prédation est souvent lente à se produire, de sorte qu'il n'est pas toujours pratique, lorsqu'il s'agit d'estimer quantitativement le régime alimentaire du prédateur, d'observer un échantillon suffisamment grand d'épisodes entiers de prédation. Heureusement, il existe dans la plupart des cas des données connexes sur la proportion de prédateurs se nourrissant de chaque type de proie. Nous démontrons comment, en divisant la proportion de tous les prédateurs se nourrissant d'un type donné de proie par le temps de contact moyen requis pour consommer un seul item de ce type de proie, on obtient un estimé de la fréquence relative de la proie dans le régime du prédateur. Une procédure analogue, fondée sur le temps de résidence dans le tractus alimentaire, donne un estimé quantitatif du régime à partir de données numériques sur les contenus stomacaux.

Received October 4, 1976
Accepted September 7, 1977

Reçu le 4 octobre 1976
Accepté le 7 septembre 1977

QUANTITATIVE knowledge of the diets of consumers is necessary for analysis of several very basic ecological considerations, including studies of ecological energetics, food webs, resource partitioning, and predator preference (e.g. Ivlev 1961; Landenberger 1968; Menge 1972a, b). To estimate a predator's diet, one can usually observe over a sufficient period of time the proportionate consumption of each prey type (e.g. morph, size-class, species, etc.). In many systems exemplified by marine invertebrate communities, however, predation proceeds so slowly that observation of several entire feeding episodes is impractical (e.g. Christensen 1970; Menge 1972a; Dayton et al. 1974). Fortunately one can often observe the fraction of all feeding predators that is found feeding upon each prey type (hereafter termed "feeding proportions"), as illustrated by Mauzey (1966), Paine (1966), Menge (1973, 1976), Dayton et al. (1977), and several others.

These feeding proportions, although related, are not equivalent to the proportions of prey types in the total diet of the predator population ("consumption" or "dietary" proportions), except where contact time per individual prey eaten is approximately the same for each prey type or where feeding is periodic such that feeding observations can be made shortly after each individual feeding predator has chosen its prey for that entire period and before any prey items have been consumed. Frequently neither of these conditions will be met. In such cases predators will tend to accumulate on those prey types that require the longest predator–prey contact times, thus leading to an overestimate of the dietary frequency of these most slowly consumed prey types. In this report we demonstrate the relationship between feeding proportions and diet, show thereby how additional data on predator–prey contact times can be used

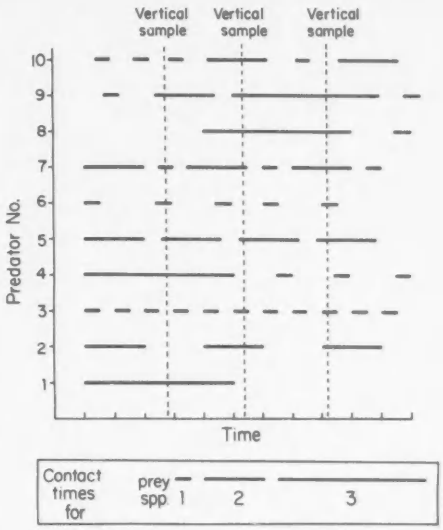
Printed in Canada (J4599)
Imprimé au Canada (J4599)

to estimate the diets of predators more accurately, and use published data to illustrate our conversion procedure.

Algebraic relationships between feeding proportions and diet — First consider the mathematically more trivial case in which the predator population is homogeneous with respect to diet, that is, a population composed of individuals that are dietary generalists to a like degree. This does not preclude preferences for certain prey types. For simplicity, let there be two prey types (types 1 and 2) of which the predators consume N_1 and N_2 total individuals during a representative period of time. We shall use X_j to represent the average period of contact required for a predator to consume one item of prey type j. Contact time, when so defined, does not encompass the total time between initiation of successive meals, but rather, includes only that period of identifiable predator–prey contact during consumption of a single prey item in the field.

It follows that during this representative period of time the total contact time between feeding predators and prey of type j will be N_jX_j. At equilibrium in a system with no feeding periodicity, the frequency of predators found feeding at any instant on species j will remain nearly constant over time and will be proportional to the relative total contact time between the predators and species j. In such an instance, the observed feeding proportions actually estimate the relative total contact times (the N_jX_j). Consequently, to convert these feeding observations to relative estimates of the predator's diet (the N_j), one must divide each observed feeding proportion by the respective average contact time per item (X_j).

Many predator populations will demonstrate some degree of feeding periodicity even if it is slight. Although for such predators feeding proportions are not likely to remain constant through time, feeding data may still be collected in such a way as to render the feeding proportions good estimates of the relative total contact times between predators and each of their prey. We suggest that feeding observations be recorded on several occasions spaced at equal intervals during the feeding cycle (or the 24-h day in the absence of detailed information on feeding cycles). The intervals between observations should usually be shorter than the average contact time required to consume the most rapidly eaten prey. In addition, an equal number of predators should be examined on each occasion regardless of whether they are actively feeding or not. Pooling of the feeding observations from the entire cycle (day) forms feeding proportions for each prey type and approximates an integration (or sum) of the total contact times between feeding predators and each of the prey types. Feeding observations gathered in this fashion should thus render them good estimates of total relative contact times (the N_jX_j); division by the average contact time per item (X_j) then yields relative estimates of the predator's diet (the N_j).



Actual Numerical Diet (Total)			Sampled Diet (Vertical Samples)		
Prey spp.	N	%	Prey spp.	N	%
1	29	63	1	5	25
2	13	28	2	9	45
3	4	9	3	6	30

FIG. 1. A hypothetical example of 10 predators feeding upon three species of prey. The feeding history of each predator is indicated for a single representative feeding period. The identity of each prey item consumed is specified by the contact time required to consume that item; contact times show a characteristic difference among species, as indicated. Actual numerical diet is calculated by pooling all acts of predation during this period. Each vertical sample reflects the activity of all predators at an instant in time. Data pooled from vertical samples constitute "feeding proportion" observations, which are biased estimates of actual numerical diet. Division of each feeding proportion by the species-specific contact time yields estimates of the relative frequency of prey in the actual diet of the predator population.

This procedure for quantitatively estimating diets can also be applied to strongly periodic predators. Alternatively, if feeding predators can be examined just after each animal has selected its prey for that feeding period and before any items have been consumed, then observed feeding proportions are directly equivalent to dietary proportions and additional work is unnecessary. Only the natural history of specific systems can provide the basis on which to choose between these methods.

Note that the algebraic conversion suggested here is independent of resting times between meals and other such activities of predators. It is based upon only the total numbers consumed (the N_j), the average contact times per item consumed (X_j), and the total contact times necessarily required for those consumption rates

(the N_jX_j). Consequently, the conversion is tautologically true, independent of the activities of nonfeeding predators.

As an illustration, consider a system composed of a population of 10 predators feeding upon three prey species (Fig. 1). Assume that the contact time required for a predator to consume a single prey item differs among the three prey species such that a single consumption of species 1 requires one-half unit of contact time, species 2 two units, and species 3 five units. Figure 1 illustrates the feeding history of these 10 predators during a representative feeding period. A series of feeding observations is represented on Fig. 1 as a vertical sample, "stopping" (as in a photograph) the feeding activity at a point in time. If the three vertical samples were taken, as indicated, at approximately equal intervals during the feeding cycle, the sampled diet (the feeding proportions) would differ greatly from the actual numerical diet for that period. However, division of the feeding proportion of each prey species by the characteristic contact time required to consume an item of that species serves to estimate the true numerical diet. In this example, dividing 25% by 0.5, 45% by 2.0, and 30% by 5.0, after rescaling to 100%, yields dietary estimates of 64, 29, and 8%. These differ only slightly from the true values of 63, 28, and 9%.

Many predator populations are heterogeneous, composed of individuals of different phenotypes, each taking characteristically different frequencies of the various prey types. If the phenotypes can be visually distinguished, then this more general representation of the predator population simply collapses into a treatment of several separate homogeneous predator populations (phenotypes). If, however, the phenotypes cannot be distinguished by inspection, a more general means of estimating total diet is required.

Assume again only two prey types and also only two predator phenotypes. Let N_{ij} equal the number of individuals of prey type j consumed by predator type i over a representative period of time. Then if X_{ij} represents the average contact time required for predator i to consume one item of prey j, the weighted mean contact time per prey item eaten is for prey type 1

$$\frac{X_{11}N_{11} + X_{21}N_{21}}{N_{11} + N_{21}},$$

and for prey type 2

$$\frac{X_{12}N_{12} + X_{22}N_{22}}{N_{12} + N_{22}}.$$

If feeding observations are taken in the fashion that we have suggested, then the observed frequency of all predators found feeding (the feeding proportion) on prey j will again be proportional to the total contact time between predators and prey j: for prey 1

$$X_{11}N_{11} + X_{21}N_{21},$$

and for prey 2,

$$X_{12}N_{12} + X_{22}N_{22}.$$

Thus, one must again divide these feeding proportions by the weighted mean contact times per item to produce estimates of the desired relative frequencies of prey in the predator's diet, $N_{11} + N_{21}$ and $N_{12} + N_{22}$.

Application to gut content data — We have developed the above relationships between feeding proportions and predator diet for those species of predators that maintain external contact with their prey during the predation process, such as predatory gastropods and those asteroids that evert their stomachs around their victims. In addition, our formal analysis has a direct analogue among those predators that completely ingest their prey. In such cases, different species of prey can possess characteristically different "residence" times in the gut of the predator. The residence time would be defined as the period of time during which the prey item remains recognizable in the gut. Those prey species with characteristically long average residence times will tend to be overrepresented among the gut contents. Since residence time in the gut is conceptually equivalent to contact time in our algebraic treatment of predation, the relative frequency of each prey species in the diet of an "ingesting" predator can be estimated from data on gut contents by using the procedure that we have recommended above.

Christensen (1970) presents data on gut contents of an asteroid, *Astropecten irregularis*, which digests prey internally. He also measures the average residence time in the gut for several of its common bivalve prey. Although residence time was not measured for all prey species, sufficient numbers are presented to permit illustration of our adjustment procedure with field data. Table 1 presents the pooled gut contents from 322 starfish (taken from Christensen's tables 7 and 13) and includes only those prey species for which Christensen also gives estimates of average residence times in the gut (Christensen's table 19). Different size-classes are treated separately for several species because residence time varies with size (Table 1). We adjust the gut content data by dividing the gut frequency of each prey species by its average residence time. These estimates of dietary proportions after conversion to percentages (in column 5) can then be compared with the original data on frequencies in the gut (in column 2).

Table 1 reveals that average residence time in the gut can differ greatly among prey species; the shortest residence time (6 h) differs from the longest (350 h) by a factor of more than 50. Consequently, errors of this magnitude could result from ignoring the potential bias of differential residence time in the gut. For instance, without adjusting the gut data for differences in average residence time, the numerical importance of *Venus gallina* in the *Astropecten* diet is apparently similar to that of *Spisula subtruncata*. However, the residence times differ by a factor of more than 6, so that in the actual diet the starfish takes about 10 *Spisula* individuals for every 1 *Venus*. Christensen (1970, p. 79–80) realizes that this bias exists in gut content data

TABLE 1. Data taken from Christensen (1970) on the bivalve gut contents of a sample of 322 *Astropecten irregularis* collected off Frederikshavn, Kattegat. The only prey data reproduced here are those for which Christensen also measured the residence time in the gut.

Prey species (size range, mm)	Total no. specimens in guts[a]	(A) % all items in guts	(B) Avg. residence time in gut[b] (h)	(A/B) Relative frequency in total diet	% all items in diet
Nucula nitida (5.5–11.3)	9	0.36	64	0.0056	0·08
Cyprina islandica (6.7–10.8)	1	0.04	45	0.0009	0.01
Venus gallina (0.8–1.2)	366	14.57	61	0.2389	3.36
V. gallina (3.2–5.8)	446	17.75	88	0.2017	2.84
V. gallina (6.2–12.5)	46	1.83	237	0.0077	0.11
Cultellus pellucidus (4.8–8.5)	12	0.48	6	0.0800	1.12
Spisula subtruncata (0.8–1.3)	550	21.89	9	2.4322	34.20
S. subtruncata (2.8–4.2)	802	31.93	9	3.5478	49.88
S. subtruncata (10.0–19.8)	8	0.32	22	0.0145	0.20
Corbula gibba (0.8–1.0)	248	9.87	17	0.5806	8.16
C. gibba (6.2–8.7)	24	0.96	350	0.0027	0.04
Totals	2512	100.00		7.1126	100.00

[a]From Christensen (1970) tables 7 and 13; for purposes of our analysis individuals falling outside the indicated size range were assigned to the nearest size category for which residence time was actually measured.

[b]From Christensen (1970) table 19.

and suggests, as we do, that an adjustment should be made by dividing the frequency of each species in the gut by its average residence time.

Our procedure for correcting gut content data can also be applied to vertebrate predators. Measurement of residence times in the gut, however, is made relatively easy for asteroids feeding on bivalve prey, because residence in the gut is terminated when the starfish ejects the emptied shells. Consequently prey residence times can be measured without killing the predators. For vertebrate predators, residence time in the gut can be measured only by killing the animals or, if possible, by stomach pumping at varying intervals after feeding until a time is found when the prey item is no longer recognizable. Nevertheless, for careful comparison of the feeding overlap between several predatory species or for detailed studies of predator preference, the added difficulty might often be justified even for vertebrates.

Discussion — The frequent lack of equivalence between feeding proportions and dietary proportions has not been widely recognized, and as a result, many studies of marine invertebrates continue to use or suggest use of feeding proportions without adjustment as a quantitative approximation of a predator's diet. Such usage of feeding proportion data results in little or no bias when applied to those invertebrates displaying strong feeding periodicity like many intertidal species responding to tidal fluctuations, for example many intertidal populations of starfish (Feder 1959; Mauzey 1966; Paine 1966, 1974; Menge 1972a, b) and predatory gastropods (Connell 1961; Paine 1963, 1966; Menge 1976). For less rhythmic feeders where observations cannot readily be made just after all feeding animals have chosen their prey for that period and before any consumption has occurred, the use of feeding proportions to estimate quantitative diets can result in larger biases. Subtidal populations of invertebrate predators are more likely to lack such discrete periodicity in feeding, such as the asteroids studied by Mauzey et al. (1968), Dayton et al. (1974), and Dayton et al. (1977) and the sea pen predators examined by Birkeland (1974). Some intertidal feeders also appear to lack sufficient periodicity, as illustrated by *Urosalpinx cinerea* studied by Wood (1968) and *Thais armigera* studied by Menge (1973).

Published solutions to the problem of accurately estimating a predator's diet from field observations have tended to be applicable only to a specific predator–prey system. Although Mauzey et al. (1968) recognized that feeding proportions are not equivalent to dietary proportions, they did not suggest any general solution to this problem. Menge (1974) also was aware of this difficulty; she, however, was able to solve the problem for her system of predatory gastropods by careful separation of prey into groups depending upon how much drilling and digestion had taken place. This process permitted her to estimate how many of the prey items being consumed had been selected during the most recent feeding period and how many represented older acts of predation. This solution will doubtless work for other systems also, but it does require the elaborate and time-consuming examination and classification of each prey item. Menge and Menge (1974), also aware

of this potential problem, suggested that one could record only those feeding choices of "actively foraging" predators. Such a solution seems feasible for predators with a strict feeding periodicity, such as the intertidal starfish they studied. For predators with less distinct feeding rhythms, one would need to make continuous observations during complete 24-h periods to achieve a representative sample of all prey items chosen. Connell (1961) also adjusted feeding proportion data in a way that was especially appropriate for his system of intertidal predatory gastropods. He assumed that feeding proportions in the absence of predator preference would reflect the relative area covered by each of two prey types, two size-classes of barnacles. This solution is unlikely to apply to predator–prey systems in general.

The correction process proposed here, which is an elaboration and formalization of Christensen's (1970) suggestion, seems to be a more general solution to this problem. It is especially useful for predators lacking discrete feeding periods, such as many subtidal invertebrates, but it can also be applied to periodically feeding predators. Our algebraic treatment reveals that the solution to the problem of accurately estimating diets is the same for a homogeneous and, more generally, a heterogeneous predator population. For both types of predator populations, one must estimate feeding proportions by pooling observations made on repeated occasions so as to render the observed feeding proportions good estimates of relative total contact times between feeding predators and each of their prey (as in Fig. 1) and divide these observed feeding proportions by the respective average contact times per item to produce estimates of the relative frequencies of prey in the predator's diet. For heterogeneous predator populations, making good estimates of the weighted average contact times can often be difficult because a long, continuous observation period is necessary to accumulate a representative sample of all consumptions and application of the weighted averages obtained under one set of conditions to another different location requires that the proportions of different predator types feeding on each prey type

$$\frac{N_{21}}{N_{11}} \text{ and } \frac{N_{22}}{N_{12}}$$

remain constant in space. Such constancy is unlikely, so that one should actually sample the duration of per item contact times from several local areas to calculate their variances before using them.

Despite the difficulties inherent in the correction processes, attention to this problem is clearly justified by the magnitude of the potential error made when contact times (or residence times in the gut) are ignored completely. For instance, the data of Christensen (1970) used in Table 1 reveal that residence times can differ by a factor of 50 or more. Menge (1972a) also estimated contact times for a starfish and its prey that differ by more than an order of magnitude among different prey species. These differences among prey in average contact times reflect the magnitude of the errors that could result in estimating numerical diets without adjusting for the contact time biases.

Note that although the method described here permits a field estimate of a predator's diet, such an approach totally ignores the responses of individual predators. The prey preference of an individual, the effects of past dietary history (learning), and other relevant individual behavioral responses are unknown. When considered important, the behavior of individuals must be studied by other techniques, such as tagging in the field or the controlled, laboratory experiments of Landenberger (1968), Birkeland (1974), and Murdoch (1969).

Acknowledgments — We thank R. Black, J. H. Connell, J. Kastendiek, S. A. Levin, B. A. Menge, W. W. Murdoch, S. Shipman and referees for helpful suggestions on this manuscript. W. Nelson provided a useful reference and B. A. Menge proposed the inclusion of Fig. 1.

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Unusual Response of a Cupric Ion Electrode in Prairie Lake Waters

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BARICA, J. 1978. Unusual response of a cupric ion electrode in prairie lake waters. J. Fish. Res. Board Can. 35: 141–143.

The Orion cupric ion electrode exhibited the theoretical (Nernstian) response of 29–30 mV per concentration decade in deionized water solutions, but in certain moderately alkaline and moderately saline natural waters of the Canadian prairies (pH 7.8–8.6, specific conductance 950–6550 μS/cm) the response to added cupric ion of over 100 μg Cu/L was twice as great. The electrode was, nevertheless, usable in natural waters provided that calibration was made with water from the same source.

Key words: water analysis (chemical), cupric ion electrode, copper, hard water

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L'électrode à ion cuprique d'Orion montre la réaction théorique (de Nernst) de 29–30 mV par 10 unités de concentration en solutions aqueuses désionisées. Par contre, dans certaines eaux naturelles modérément alcalines et modérément salées des Prairies canadiennes (pH 7.8–8.6, conductance spécifique 950–6 550 μS/cm) la réponse à l'addition d'ion cuivrique de plus de 100 μg Cu/L est deux fois plus forte. On peut quand même utiliser l'électrode dans des eaux naturelles pourvu qu'elle soit calibrée avec une eau provenant de la même source.

Received February 4, 1977
Accepted September 21, 1977

Reçu le 4 février 1977
Accepté le 21 septembre 1977

DETERMINATION for copper in natural waters by conventional absorptiometric or atomic absorption methods gives a total copper concentration that may be meaningless or only marginally useful in toxicity studies. It is the free cupric ion Cu^{2+} that is assumed to be toxic to fish and other aquatic organisms (Lloyd 1965; EIFAC 1976). Most of the copper added to hard water appears to be complexed by various inorganic components or is eventually removed from solution by precipitation as $Cu(OH)_2$ or $CuCO_3 \cdot Cu(OH_2) \cdot H_2O$.

The Orion cupric ion electrode Model 94-20 is a solid state device designed for the range 10^{-7} to 1 M (Cu^{2+}). Its potential is claimed to be proportional to the logarithm of the activity of free cupric ion, for which it is selective (Orion Research Inc. 1975), and follows the Nernst equation:

$$E = E_o + \frac{2.3RT}{nF} \log a$$

where E is the potential developed by the cupric ion electrode, E_o a constant for unit activity of cupric ion, R the gas constant, T absolute temperature, F the Faraday constant, n change of the ion, and a ionic

Printed in Canada (J4716)
Imprimé au Canada (J4716)

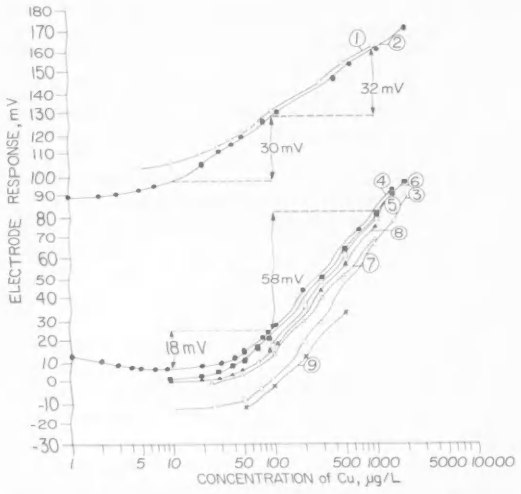

FIG. 1. Electrode response to different concentrations of added copper in different natural waters. 1 — deionized water (pH 6.4–3.8 after addition of standard solution); 2 — deionized water + ISA ($NaClO_4$); 3 — Lake 811 (6550 µS/cm, 229 mg/L Ca, 786 mg/L Mg, 408 mg/L HCO_3, pH 7.90; 4 — Lake 811 + ISA; 5 — Lake 255 (950 µS/cm, 62.8 mg/L Ca, 101 mg/L Mg, 239 mg/L HCO_3, pH 8.50); 6 — Lake 255 + ISA; 7 — Lake 885 (1460 µS/cm, 43.4 mg/L Ca, 151 mg/L Mg, 378 mg/L HCO_3, pH 8.40); 8 — Lake 885 + ISA; 9 — usual 3-point calibration curve used for routine determinations.

activity for cupric ion. In dilute solutions, concentration is generally equal to activity. For bivalent cupric ion the change in potential produced by a tenfold change in concentration is 26.6 mV at 25°C; for a univalent ion this is 59.2 mV. This holds down to about 10^{-6} M; at lower concentrations the sensitivity decreases. It is therefore better, at the lowest concentrations, to use a calibration curve, from a series of concentrations, rather than the known addition method can be used in the linear part of the relationship. The addition of $NaClO_4$ to 0.1 M as ionic strength adjuster (ISA) is recommended to keep ionic strength of samples and standards approximately equal.

This report describes some observations on an unusual response of the cupric ion electrode in hardwater systems of the Canadian prairie lakes to bring it to the attention of workers using the electrode and avoid misinterpretation of measured data.

Materials and methods — Apparatus — Cupric ion electrode (Orion model 94-20); reference electrode, Ag/AgCl type (Orion model 90-01 single-junction reference electrode); millivoltmeter (Orion model 801A digital pH meter); magnetic stirrer; Eppendorf pipettes, 10, 20, 50 µL.

Solutions — Standard copper solution: cupric nitrate 1 mL = 1000 µg Cu^{2+} (pH < 4); dilute 10 or 100× for lower concentrations. ISA, 10 M sodium perchlorate.

Procedure — Use exactly 100 mL of sample in a 150-mL beaker and introduce both electrodes. Stir moderately. Add known increments of standard Cu solution using Eppendorf pipettes. Wait 2–3 min after each addition and record the potential. Construct the calibration curve by plotting the potentials vs. concentrations on a semilog paper (Fig. 1). For a sample of unknown concentration, record the electrode potential in about 100 mL of sample and read the concentration from the calibration curve. Whenever possible use water from the same source to construct calibration curve (i.e. copper-untreated part of a lake, tank, make-up water, etc.). Otherwise, add 1 mL ISA to offset difference in ionic strength between samples and standards.

Use of the electrode in natural hard waters — During 1975 and 1976 the cupric ion electrode was used in experiments on the effects of copper on fish and algae in seven lakes (Whitaker et al. 1978; Majewski, Freshwater Institute, Winnipeg, personal communication). The waters had specific conductance of 950–6550 µS/cm and initial pH values of 7.8–8.6. They also contained bicarbonate from 239 to 408 mg/L HCO_3^- and appreciable amounts of potentially complex-forming ammonia and humic acids. From data on stability constants (Sillén and Martell 1964) they might be expected to bind free copper, causing a low or zero response to any copper added for calibration. In fact, this is not what occurred.

In deionized water (Fig. 1, lines 1 and 2) with pH less than 6 and all added copper in Cu^{2+} form, electrode response is close to the theoretical slope of 30 mV/decade of concentration, in both 10–100 and 100–1000 µg Cu/L ranges. Addition of ISA did not significantly alter the slopes at these concentrations but expanded the working range down to about 5 µg/L Cu.

Given these results it could be expected that calibration curves (lines 3–8) for hard water containing natural chelating agents would be flattened, with slopes less than 30 mV. This is more or less true in the range 10–100 µg Cu/L where slopes were 13–22 mV. At higher concentrations, however, slopes were 56–68 mV, twice what is expected, and this was found consistently, not only in the experiments reported here, but in all of over 200 measurements on different lakes, during 2 yr. Addition of ISA did not affect the slope, though it caused a small potential shift similar to those common with specific ion electrodes, which necessitates daily recalibration. In natural waters the working range started at about 20 µg Cu/L.

An explanation of this unexpected behavior of the cupric ion electrode is beyond the scope of this note and warrants further investigation. Obviously the electrode responds to other unknown factors. Possible explanations include singly charged Cu complexes like $CuOH^+$ or $CuHCO_3^+$ that can exist in alkaline waters (Sylva 1976) and cause doubling of the slope: or an effect of the drop in pH (0.2–0.3 units of pH in lake

water and up to 3.0 units in deionized water) during additions of the acid standard solution (Sekerka, Canada Centre for Inland Waters, Burlington; Wagemann, Freshwater Institute, Winnipeg; personal communication). Thus absolute readings of "free" ionized copper by the electrode are questionable and the data correspond rather to "electrode detectable" copper analogous to "labile" copper as described by Chau and Lum-Shue-Chan (1974). Despite this the electrode is a useful tool in monitoring relative changes in concentration of copper added to lakes or tanks over a period of time in algae and fish toxicity studies.

Acknowledgments — I am gratefully indebted to Drs *F. A. J. Armstrong, R. Wagemann, I. Sekerka,* and *Y. K.* Chau for critical comments on the manuscript, and to J. *Gibson, M. Buckley, and H. Majewski for their laboratory* and field assistance. Measurements of total copper by *atomic absorption spectrophotometry were done by B. Hunt* and A. Lutz.

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Technique for Tagging Small Fish with I^{131} for Evaluation of Predator–Prey Relationships

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FITZGERALD, G. J., AND M. H. A. KEENLEYSIDE. 1978. Technique for tagging small fish with I^{131} for evaluation of predator–prey relationships. J. Fish. Res. Board Can. 35: 143–145.

Experiments to test the feasibility of using radioactive I^{131} as a technique for evaluation of conspecific predation on fry by adult convict cichlids (*Cichlasoma nigrofasciatum*) are described. The technique is useful for short-term quantitative studies of predator–prey interactions where it is not possible to observe and quantify predation directly or where stomach content analysis of adults is not feasible.

Key words: radioactive tagging, predator–prey interactions, *Cichlasoma nigrofasciatum*

FITZGERALD, G. J., AND M. H. A. KEENLEYSIDE. 1978. Technique for tagging small fish with I^{131} for evaluation of predator–prey relationships. J. Fish. Res. Board Can. 35: 143–145.

Nous décrivons des expériences visant à vérifier la faisabilité d'une technique utilisant l'isotope radioactif I^{131} pour l'évaluation de la prédation des adultes aux dépens des alevins de *Cichlasoma nigrofasciatum*. Cette technique s'est avérée utile pour les études quantitatives à court terme des interactions prédateur–proie, lorsqu'il est impossible d'observer et de quantifier directement la prédation ou d'analyser les contenus stomacaux des adultes.

Received May 12, 1977
Accepted October 12, 1977

Reçu le 12 mai 1977
Accepté le 12 octobre 1977

ALTHOUGH the use of radioisotopes for marking fish is now well established (e.g. Scott 1961, 1962; Hoss 1967; Carlson and Shealy 1972; Peters and Hoss 1974), radioactive tags have seldom been used to document predation. An exception is Carlson and Shealy (1972), who found that radioactive tagging was suitable for studies

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Printed in Canada (J4828)
Imprimé au Canada (J4828)

of invertebrate predators on prolarval largemouth bass.

This note describes a technique for tagging young free-swimming convict cichlids (*Cichlasoma nigrofasciatum*) for evaluation of conspecific predation on fry by adults (FitzGerald 1976). We were interested in identifying which particular fish in a small pool ate the young fish. Neither visual observation of predation nor stomach content analysis was feasible in this study.

The technique involved labeling fry with radioactive I^{131} and later measuring the level of radioactivity in adult fish held in the same container. This isotope was selected for several reasons. It is readily absorbed from the animal gut, most of it ending up in the thyroid gland. It has a short half-life (8.5 days) and a strong rate of gamma emission (Thornburn 1972). These factors were important for safety reasons and because a very dilute system was used (food-fry-adults).

Preparation of labeled food — A commercially prepared radiopharmaceutical, I^{131}-Triolein (glyceryl trioleate; Mallinckrodt Chemical Works, St. Louis, Mo.) was used to tag the food (egg yolk). It is one of the chief constituents of oils and fats of animal and vegetable origin.

The procedure was to aggregate 1 mL of egg yolk with 1 mCi of tagged Triolein at 80°C with mortar and pestle. The supernatant was then centrifuged. Preparation and storage of tagged food and all radioactivity measurements were done in the Department of Nuclear Medicine, University Hospital, University of Western Ontario. Feeding of fry, several pilot studies, and the use of the technique as part of a larger study (FitzGerald 1976) were done in a special Zoology Department radioactivity laboratory. A multichannel sodium–iodide scintillation (Tobor) counter was used to record radioactivity. Five replicate measures of radioactivity (in counts per minute) were taken on all samples of food, fry, adults, and holding water. All counts were corrected for background radiation levels and natural decay rate.

The tagging of fry and their introduction into pools containing adult fish presented several technical and logistical problems. A series of investigations was performed to determine (1) whether fry would eat the tagged food, (2) the length of time that detectable amounts of radioactivity remained in the fry, (3) the behavior of tagged fry in comparison with untagged controls, (4) whether adult fish would consume tagged fry, and (5) the length of time that detectable amounts of radioactivity remained in the adults.

Tagging the fry — Fry were tagged as follows: approximately 200 5–7-day-old fry were placed in a small aquarium (40 × 26 × 22 cm) where they were given excess quantities of radioactive food and allowed to feed for 24 h. There were no readily observable differences in feeding and locomotor behavior between tagged fry and controls. They were then captured by dip net, rinsed twice in aged, uncontaminated water and placed in a second small aquarium containing nonradioactive water. At 6-h intervals, beginning immediately after removal from the feeding tank and ending 48 h later, 15 fry were removed by dip net, rinsed, and dried of excess water. They were then placed in a sterile, uncontaminated plastic test tube and their radioactivity levels measured.

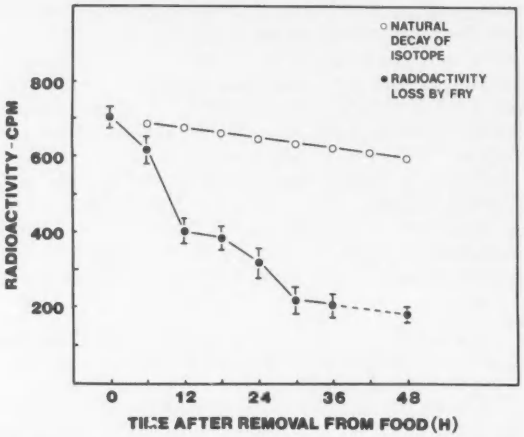

FIG. 1. Change in radioactivity of *C. nigrofasciatum* fry measured at 6-h intervals beginning immediately after removal from tank containing radioactive food. Solid circles are means (± 1 SD) of five measurements (counts per minute, CPM) taken on separate samples of 15 5–7-day-old fry. No data available at 42 h. Open circles show natural decay rate of the I^{131} isotope.

Our data (Fig. 1) show that radioactivity in the fry could be measured for at least 48 h after removal from labeled food. If longer counting periods (e.g. 5 rather than 1 min) had been used, the length of time the tag could be detected would be increased.

Tagging the adults — Three adult *C. nigrofasciatum* (9.0–10.0 cm total length) were selected from a stock population. Each was placed in a separate small aquarium and given access to 15 tagged fry. These samples of 15 fry had a mean count of 702 counts/min when given to adults. The number of fry consumed by each adult within 24 h was recorded and the radioactivity level of each adult then determined.

The adult fish were removed from their aquaria and placed in a jar of uncontaminated water. Each animal was rinsed again in tap water, wrapped in wet paper towel, placed inside a small plastic bag, and finally placed in the Tobor scintillator counter. Upon return to the laboratory they showed no apparent ill effects from the handling and counting procedures. Mean counts per minute (±1 SD) of five counts on each fish (Table 1) show that adult fish are detectably radioactive for at least 24 h after consuming tagged fry.

The technique described here is useful for short-term quantitative studies of predator–prey interactions where it is not possible to observe and quantify predation directly or where the killing of adult animals for stomach analysis is not possible. The technique may also be useful in tagging aquatic organisms for other purposes where an easily detected but short half-life isotope is needed.

TABLE 1. Radioactivity of adult *C. nigrofasciatum* after consuming tagged fry. Data are means (±1 SD) of five measurements (in counts per minute) corrected for background radiation and natural decay of the isotope.

Subject no.	Radioactivity	No. fry consumed
1	656±89	15
2	696±61	15
3	617±42	13

Acknowledgments — The technical expertise of Mr P. McKinnie was essential to the development of the technique. Dr M. L. Chamberlain suggested the procedure for tagging fry and permitted us to use the facilities of the Department of Nuclear Medicine. The advice of Dr B. G. Atkinson is greatly appreciated. The work was supported by a National Research Council Grant to M. Keenleyside.

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Variation in Age at First Spawning in Atlantic Salmon (*Salmo salar*)

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NÆVDAL, G., M. HOLM, O. INGEBRIGTSEN, AND D. MØLLER. 1978. Variation in age at first spawning in Atlantic salmon (*Salmo salar*). J. Fish. Res. Board Can. 35: 145–147.

Significant differences in the proportion of mature fish in groups of Atlantic salmon (*Salmo salar*) of different river origins maintained under the same conditions were observed during the 1st and 2nd yr of sea rearing. Nearly all fish in sib groups originating from grilse rivers matured during the 2nd sea year, while few or no mature fish were found in the groups originating from rivers producing mainly multi-sea-winter salmon. The observations were in accordance with the life histories of the different river populations, indicating that salmon inherit rather than acquire a tendency to mature at a certain age.

Key words: Atlantic salmon, maturation age, cultured salmon, river populations

NÆVDAL, G., M. HOLM, O. INGEBRIGTSEN, AND D. MØLLER. 1978. Variation in age at first spawning in Atlantic salmon (*Salmo salar*). J. Fish. Res. Board Can. 35: 145–147.

Nous avons observé durant la 1re et la 2e a d'élevage en mer des différences significatives dans la proportion de poissons mûrs dans des groupes de saumons atlantique (*Salmo salar*) provenant de différentes rivières et maintenus dans des conditions identiques. Presque tous les poissons de progénitures provenant de rivières à grilse atteignent la maturité au cours de leur 2e a en mer, alors qu'on trouve peu ou pas de poissons mûrs dans les groupes provenant de rivières qui produisent en grande partie des saumons de plusieurs hivers en mer. Ces observations sont conformes au cycle biologique des populations des différentes rivières, ce qui indique que la tendance à atteindre la maturité à un certain âge est héréditaire et non acquise chez le saumon.

Received October 12, 1976
Accepted September 1, 1977

Reçu le 12 octobre 1976
Accepté le 1 septembre 1977

Printed in Canada (J4608)
Imprimé au Canada (J4608)

ATLANTIC salmon (*Salmo salar*) has a wide distribution on both sides of the North Atlantic. Each river has its own discrete population, and sometimes more than one population is present in the same river system (Møller 1970, 1971). Considerable variation in age and size at first maturity of salmon exists within and among populations. Ritter (1972, 1974) emphasized that presmolt growth rate has an influence on this trait because fast growing parr and, consequently, young smolts produce a relatively low proportion of grilse (maturing and mature 1-sea-winter salmon). However, he also mentioned that other factors, such as heredity, seem to have an influence on age at first maturity. Elson (1971) observed that when grilse were used as parent fish, the offspring were largely grilse, but when virgin 2-sea-winter salmon were used, comparatively few grilse were produced.

In the present paper observations on sea age and size at first maturity of Atlantic salmon under fish farming conditions are presented and discussed in relation to the life histories of the river populations from which the parent fish originated.

Materials and methods — A program for the genetic improvement of Atlantic salmon for fish farming in Norway was started in autumn 1971. Fertilized eggs for four sib groups were collected from each of the following rivers: 12 Norwegian, 2 Canadian, and 1 Swedish. Eggs were also obtained from the Eros Laks commercial fish farm, Bjordal, Norway. Normally two fishes of each sex were used from each river. The eggs of each female were divided into two parts, each of which was fertilized by one male, thus resulting in four groups from each river. The Eros Laks farm provided 12 groups of eggs fertilized according to same mating pattern.

The newly fertilized eggs arrived at Matredal station for incubation from end of October until beginning of January 1972. The first groups to arrive hatched in April 1972 and the last ones in the beginning of June. Mean hatchery time was approximately 5 mo and 3 wk. The Canadian groups came to the station as eyed eggs in March and they hatched shortly after arrival. The salmon were reared in cylindrical 1.7-m^3 fiber glass tanks (depth 1 m) from the swim-up fry stage until 16 mo old. As far as possible a similar environment was maintained in all tanks. As the salmon grew larger the groups were reduced to about 2000 fish/tank. To get a random sample when reducing the size of the groups, the fish were crowded in a small container and then sampled with a dip net. Accidents with the water flow and disease attacks eliminated some groups and drastically reduced the numbers in others.

In August 1973 the parr were transferred to 18-m^3 floating pens in brackish water ($\approx$ 15‰ salinity) near the station, and the groups were reduced to a maximum of 600 individuals/group. Sib groups from the same river were marked by removal of adipose and/or pelvic fins and stocked in the same pen.

Most of the parr reached smolt size in 2 yr. The fish were classified as smolts by external examination; fish classified as smolts were $\approx$ 12–17 cm. In June 1974 the fish were transferred to full strength seawater. Twenty smolts from each surviving group were tagged with numbered tags (Carlin 1955) and stocked in a 100-m^3 quadrangular floating pen at a fish farm belonging to Svanøy Stiftelse, Svanøybukt, Norway, 200 km north of the research station. The remaining fish in each group were transferred to 50-m^3 pens at Risnefisk commercial fish farm, Brekke, Norway, $\approx$ 30 km from the station.

In April 1975, 100 fish in each group were tagged with numbered FT-4 Floy tags (Floy Tags & Manufacturing, Inc., Seattle, Wash.) and transferred to 500-m^3 octagonal floating pens at the fish farm of Svanøy Stiftelse. The remaining fish (maximum of 50 individuals/group) were also tagged with Floy tags and retained at the Risnefisk farm. Thus, depending upon the number of individuals in a sib group, the groups were divided and reared under one to three regimes. Although rearing and environmental conditions varied with the regime, the groups reared under a particular regime were maintained under the same conditions.

Total lengths were recorded every 6 mo in spring and autumn and individual weights in spring and autumn 1975. Maturation was determined by external examination (brownish color, hooked underjaws, running milt).

Differences in the incidence of mature fish among sib groups were tested for significance by chi-square (Snedecor and Cochran 1956). Relationship between maturation and growth was tested through correlation analysis. Before calculating coefficient r values percent data were transformed to their inverse sine.

A more detailed description of the chemical and physical properties of the water and of the survival and growth of the different sib groups during early rearing is given in a report by Nævdal et al. (1975).

Results and discussion — The proportion of fish maturing in the 1st sea year varied among sib groups with the highest proportion present in groups originating

TABLE 1. Percent mature fish in the 1st and 2nd yr, by river origin, for salmon reared in floating pens in the sea. Figures in parentheses represent proportion of mature fish that were males.

Origin	1st yr		2nd yr	
	No. fish	% mature	No. fish[a]	% mature
Lakselv	43	0	29	10 (33)
Altaelv	11	0	9	0
Målselv	246	6 (100)	181	22 (70)
Rauma	118	0	87	89 (60)
Gaula	358	2 (100)	281	2 (80)
Lærdalselva	290	0	257	3 (86)
Vosso	447	3 (100)	348	4 (62)
Longevågselva	86	1 (100)	70	96 (63)
Eio	119	1 (100)	102	13 (79)
Opo	180	0	150	0
Etne-elva	353	0	311	4 (42)
Tengselva	187	0	166	27 (80)
Skellefteälv, Sweden	462	2 (100)	290	47 (97)
Maria Pond, Canada	169	2 (100)	130	4 (40)
McDonald River, Canada	66	28 (100)	52	63 (34)

[a]Number of fish in 2nd yr does not include fish retained at the Risnefisk farm after April of the 2nd sea year.

from salmon of McDonald River, Canada (Table 1). All the mature fish were males and resembled precocious parr 15–19 cm long.

The proportion of mature fish observed in the 2nd sea year varied significantly ($P < 0.001$) among sib groups from different river populations. In groups that originated from rivers that produce mainly grilse, such as Lonevågselva, Norway, and McDonald River, Canada, most of the individuals matured during the 2nd yr. Few or no mature fish were found in the 2nd yr in sib groups originating from rivers producing salmon that mature in the 3rd or 4th sea year, such as Altaelv, Opo, Laerdalselv, Gaula and Etne-elv. The age at first maturation of the pen-reared fish correlated well with the life histories of the different river populations.

Incidence of maturity was similar regardless of rearing history in all but a few sib groups. Within these groups salmon reared from the smolt stage at the fish farm of Svanøy Stiftelse exhibited higher proportions of mature fish than did counterparts spending all or part of their sea life at the Risnefisk farm. These differences in incidence of maturity may be attributed to differences in rearing conditions at the two farms or related to differences in the size of the smolts at the beginning of the sea-rearing phase. Because of the requirement of having each of them tagged, smolts transferred to the fish farm of Svanøy Stiftelse were larger than those sent to the Risnefisk farm.

The proportion of salmon maturing during the 2nd yr varied little among sib groups originating from the same river population, although wide variation was observed among sib groups originating from salmon of the Eros Laks farm. The salmon stock at Eros Laks originates from salmon caught in the sea and therefore is probably a composite of several different river populations. This may be the reason for the variation in the results recorded for the groups originating from the Eros Laks stock. Because of this variation and the uncertainty as to the river origin of the stock, data on incidence of maturity for sib groups from the Eros Laks salmon were not included in Table 1.

Among sib groups growth rate varied greatly, although no correlation ($r = 0.005$) was found between mean length after 1 yr in the sea and the proportion of fish maturing the following autumn. A low but significant negative correlation ($r = -0.49$, $P < 0.01$) was found between mean lengths in the autumn and proportion of fish maturing the same year. These results imply that maturation retards growth and that growth before onset of maturation is uniform regardless of the age at which salmon are destined to mature. Within groups mean lengths in the autumn were lower for maturing than for nonmaturing fish, although maturing fish were among the smallest and the largest fish.

All the fish maturing in the 1st sea year were males. Similarly in the second sea year the mature fish in most groups were predominantly males, although their proportion did vary greatly and in three groups more than one half the mature fish were females (Table 1). All the males maturing the 1st yr were kept with the other fish in their respective groups. However, since most of them were not individually tagged prior to the 2nd sea year, it is not known whether these males died during the following winter or whether they survived and did not mature again.

The present investigations suggest that the variation in age at first maturity of Atlantic salmon is largely attributed to a genetic base probably originating as an adaptation to different river conditions. Environment may have some influence on age at which salmon first mature, since fish of the same river origin, reared under different environmental conditions, gave similar but not identical results.

Acknowledgments — This work was supported by Fiskerinæringens Forsøksfond (Norwegian Fisheries Research Fund) and Norges Fiskeriforskningsråd (Norwegian Council for Fisheries Research).

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Ultrastructural Studies of Piscine Erythrocytic Necrosis (PEN) in Atlantic Herring (*Clupea harengus harengus*)

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RENO, P. W., M. PHILIPPON-FRIED, B. L. NICHOLSON, AND S. W. SHERBURNE. 1978. Ultrastructural studies of piscine erythrocytic necrosis (PEN) in Atlantic herring (*Clupea harengus harengus*). J. Fish. Res. Board Can. 35: 148–154.

Erythrocytes of PEN-positive Atlantic herring (*Clupea harengus harengus*) were examined to determine their ultrastructure. Cytoplasmic inclusions were of two types when observed under the electron microscope. The first type (type I) appeared coarsely granular, electron dense, round, and up to 1.5 μm in diameter. Virions were closely associated with this type of inclusion. The second type of inclusion (type II) had approximately the same appearance as the surrounding cytoplasm, from which it was separated by a discrete membrane, and was variable in size. Virions were not intimately associated with type II inclusions. Virions occurred singly or in clusters within the cytoplasm or in association with type I inclusions and were hexagonal and 145 nm in diameter. Virions were composed of a rigid hexagonal capsid 8 nm wide, a lighter 16-nm region, and a core 100 nm in diameter. The virus of PEN is presumptively classified as an Iridovirus.

Key words: ultrastructure, erythrocytes, virology

RENO, P. W., M. PHILIPPON-FRIED, B. L. NICHOLSON, AND S. W. SHERBURNE. 1978. Ultrastructural studies of piscine erythrocytic necrosis (PEN) in Atlantic herring (*Clupea harengus harengus*). J. Fish. Res. Board Can. 35: 148–154.

Nous avons examiné des érythrocytes de hareng atlantique (*Clupea harengus harengus*) ayant réagi positivement à la nécrose érythrocytique des poissons (NEP), afin d'en déterminer la structure ultramicroscopique. Lorsque examinées au microscope électronique, les inclusions cytoplasmiques se présentent selon deux types. Le premier (type I) a une forme grossièrement granuleuse, est opaque aux électrons, est rond et mesure jusqu'à 1.5 μm de diamètre. Des virions sont étroitement associés à ce type d'inclusion. Le second type d'inclusions (type II) a à peu près la même apparence que le cytoplasme qui l'entoure dont il est séparé par une membrane distincte, et est de taille variable. Les virions ne sont pas étroitement associés au type II d'inclusions. Les virions se rencontrent seuls ou en grappes à l'intérieur du cytoplasme ou en association avec des inclusions du type I, ont une forme hexagonale et mesurent 145 nm de diamètre. Les virions sont constitués d'une capside hexagonale rigide de 8 nm de largeur, une région plus claire de 16 nm et un centre de 100 nm de diamètre. Nous classons provisoirement le virus de la NEP dans la catégorie des Iridovirus.

Received June 20, 1977
Accepted September 19, 1977

Reçu le 20 juin 1977
Accepté le 19 septembre 1977

WITHIN the last decade, intracytoplasmic inclusions have been found in the erythrocytes of several species of marine fishes. Affected species include Atlantic cod, *Gadus morhua*, longhorn sculpin, *Myoxocephalus octodecemspinosus*, sea snail, *Liparis atlanticus* (Laird and Bullock 1969), blenny, *Blennius pholis* (Johnston and Davies 1973), alewife, *Alosa pseudoharengus* (Sherburne 1977), and Atlantic herring, *Clupea harengus harengus* (Sherburne 1973). Laird and Bullock (1969) termed the condition piscine erythrocytic necrosis (PEN), and their terminology will be followed in this report.

Ultrastructural studies of PEN-positive cod and blenny erythrocytes revealed the presence of hexagonal particles 200–300 nm diam (Walker 1971; Walker and Sherburne 1977; Appy et al. 1976; Johnston and Davies 1973) that morphologically resembled viruses of the Iridovirus group.

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Printed in Canada (J4889)
Imprimé au Canada (J4889)

In this report evidence is presented that the intraerythrocytic inclusions of Atlantic herring are associated with viruslike particles that are similar to, but morphologically distinct from, those of cod and blenny and can be presumptively classed with the Iridoviruses.

Materials and methods — All herring were captured in purse and stop seines near Boothbay Harbor, Maine, in the spring and winter 1976.

Initially blood samples were collected into fixatives for electron microscopy or onto glass slides for thin smears by severance of the caudal peduncle. Samples taken in the winter for light and electron microscopy were collected by caudal venipuncture with an unheparinized 1-cm^3 tuberculin syringe fitted with a 26-gauge, 10-mm needle.

Three fixatives were used for electron microscopy, and in each instance the fixation time was 1 h. The first was 3% glutaraldehyde in a 0.066 M phosphate buffer, pH 7.2 (Hayat 1972), to which sucrose had been added to a final concentration of 4%. The second was a marine saline phosphate buffer containing 0.185 M NaCl, 0.05 M KCl, 0.025 M Na_2HPO_4, 0.02 M NaH_2PO_4, 0.19% glucose, and 2% glutaraldehyde. The third was 2% glutaraldehyde in Eagle's minimal essential medium, modified for use with marine cell culture (Clem et al. 1965).

Following glutaraldehyde fixation the blood samples were washed, fixed in 2% OsO_4 in the appropriate buffer, embedded in 2% ionagar, and dehydrated through a graded ethanol series. Following final dehydration in acetone or propylene oxide, the samples were embedded in Spurr's medium (Hayat 1972) and sections cut on an MT-2B ultramicrotome. Sections were poststained with 2% aqueous uranyl acetate and Reynold's lead citrate for 30 and 2 min, respectively, and examined in a Phillips EM201 electron microscope at an accelerating voltage of 60 kV.

Results and discussion — Infected erythrocytes — Erythrocytes from PEN-negative herring were ultrastructurally identical to trout erythrocytes as reported by Sekhon and Beams (1969).

There was a marked alteration in the ultrastructural appearance of erythrocytes from PEN-positive herring. The surface of the affected cell was irregularly lobated and generally lost its symmetric elliptical shape. A further characteristic morphologic change was the distinct margination of the nuclear chromatin, evident as an accumulation of electron-dense material at the periphery of the nucleus with a paucity of densely staining material in the central region (Fig. 1). The nucleus also became irregular, but retained its structural integrity as shown by the continued presence of the karyotheca. Occasionally aggregates of coarsely granular material were noted in nuclei with marginated chromatin and may correspond to intranuclear inclusions seen in PEN-infected herring by light microscopy (Fig. 2).

The finding of occasional acidophilic intranuclear inclusions was unexpected, but may indicate some nuclear involvement in viral replication. No ultrastructural evidence of whole virions in the nucleus was observed. However, the large aggregates of coarse granules in the nucleus (Fig. 1) may be viral subunits or viral-induced materials which could be seen as inclusions by light microscopy.

In addition, virus-infected cells generally contained many conspicuous electron-dense granules, arranged singly or in small clumps, within the cytoplasm. These granules were approximately 20 nm diam and appeared to be dispersed evenly throughout the cytoplasm of infected cells, whereas few of these structures were observed in erythrocytes of PEN-negative herring.

Clearly normal erythrocytes, which lack ribosomes or at most contain only a few, do not have the cellular machinery to promote the production of new proteins. To produce virus, these same erythrocytes must, under the direction of the viral genome, synthesize adequate numbers of ribosomes to make possible complete viral replication. Thus, the granules seen in infected erythrocytes are likely ribosomes.

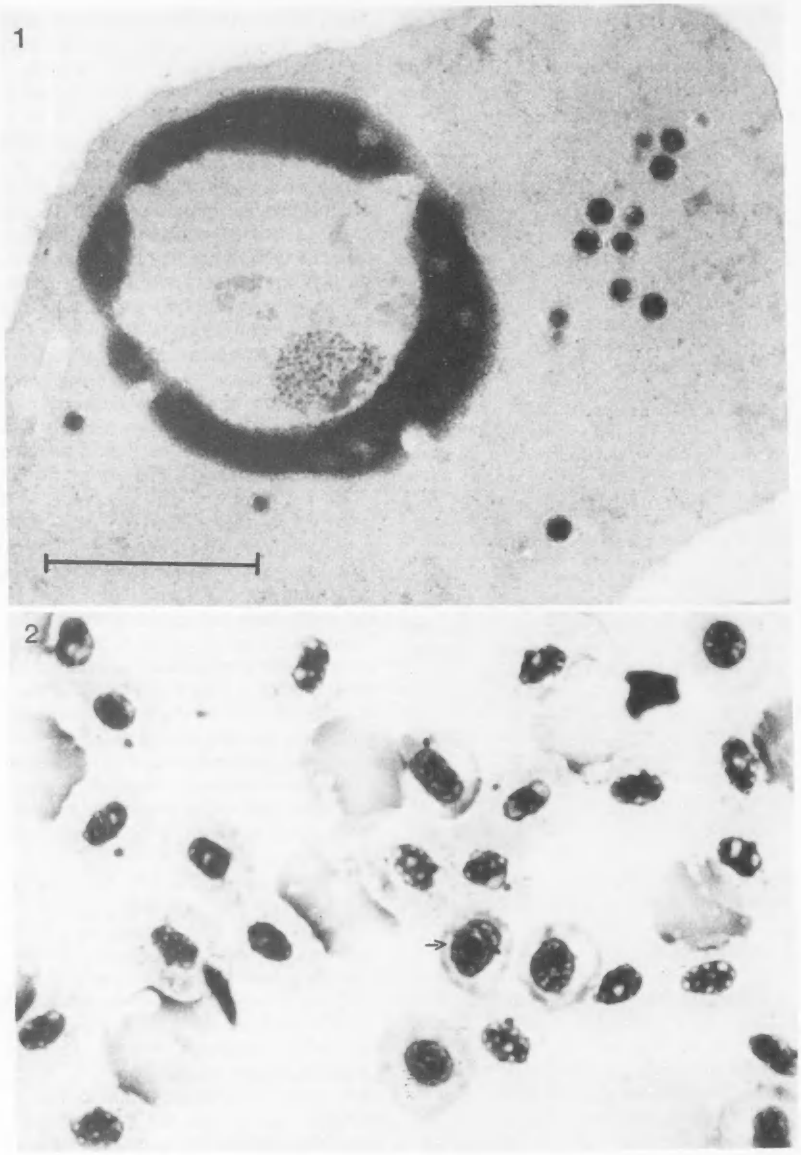
Two apparently distinct types of inclusions were noted in erythrocytes from PEN-positive fish, in approximately equal frequencies. Type I inclusions were virus associated, coarsely granular, electron dense, circular, and up to 1.5 μm diam; they occured singly in a cell and were not membrane bound (Fig. 3). In all instances viruslike particles were found in close association with the inclusions, either at the periphery or, occasionally, within the interior of the inclusion. Some of the particles appeared incomplete, and their proximity to the inclusion might indicate that they were being formed from material within the inclusion.

Rarely, lamellar bodies (Fig. 4) with alternating dark and light bands 50 nm apart, with the dark bands approximately 25 nm wide, were found near or within the inclusion.

Type I inclusions closely resemble viroplasm such as that found in PEN of cod (Walker 1971; Appy et al. 1976; Walker and Sherburne 1977) and blenny (Johnston and Davies 1973) and in the virus infections of higher vertebrates (Fenner 1974).

Type II inclusions were found only in erythrocytes of PEN-positive herring, were not virus associated, and were morphologically distinct from type I inclusions. These inclusions ranged from <0.5 to >3 μm diam and were usually multiple within a cell. They were less electron dense than the surrounding cytoplasm and were surrounded by multiple membranes (Fig. 5). Cells in which these inclusions were found did not always contain virus particles, but in all instances the nucleus had the characteristic marginated chromatin of virus-containing cells and the cellular shape was distorted. Sometimes the interior of these inclusions had the same texture as the surrounding cytoplasm, from which it is separated by a discrete contiguous membrane.

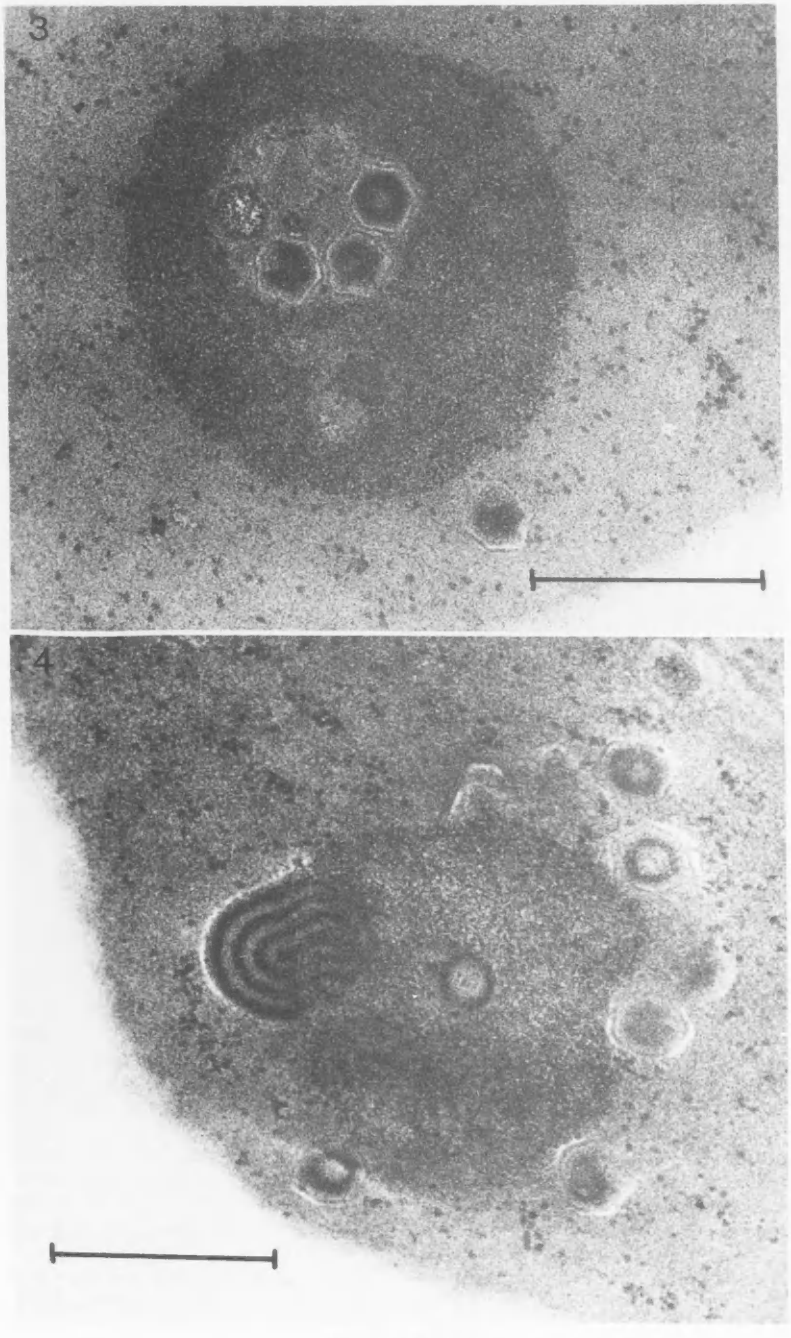
These inclusions were structurally quite unlike the type I inclusions, and their relationship to PEN is at this time unknown. It is important to know whether



FIGURES 1 AND 2

FIG. 1. PEN-infected erythrocyte showing severe chromatin margination and coarse granular aggregates in nucleus, and PEN virions. Bar represents 1 μm. FIG. 2. Giemsa stain of PEN-positive herring blood. Arrow indicates intranuclear inclusion (994×).

these inclusions are viral induced and whether they appear similar to type I inclusions after staining with Giemsa or Wright's stain, since at the present time PEN is diagnosed solely on the basis of inclusions in the cytoplasm of erythrocytes. Histochemical tests must be performed to determine whether any difference exists between the two types of inclusions at the light microscopy level. Using methyl green–pyronin Y and Feulgen stains, we have determined that the inclusions in herring erythrocytes contain DNA.



FIGURES 3 AND 4

FIG. 3. Type I inclusion with PEN virions at the periphery and in the interior of inclusion. Bar represents 1 μm. FIG. 4. Type I inclusion surrounded by virions and containing a marginal lamellar body. Bar represents 1 μm.

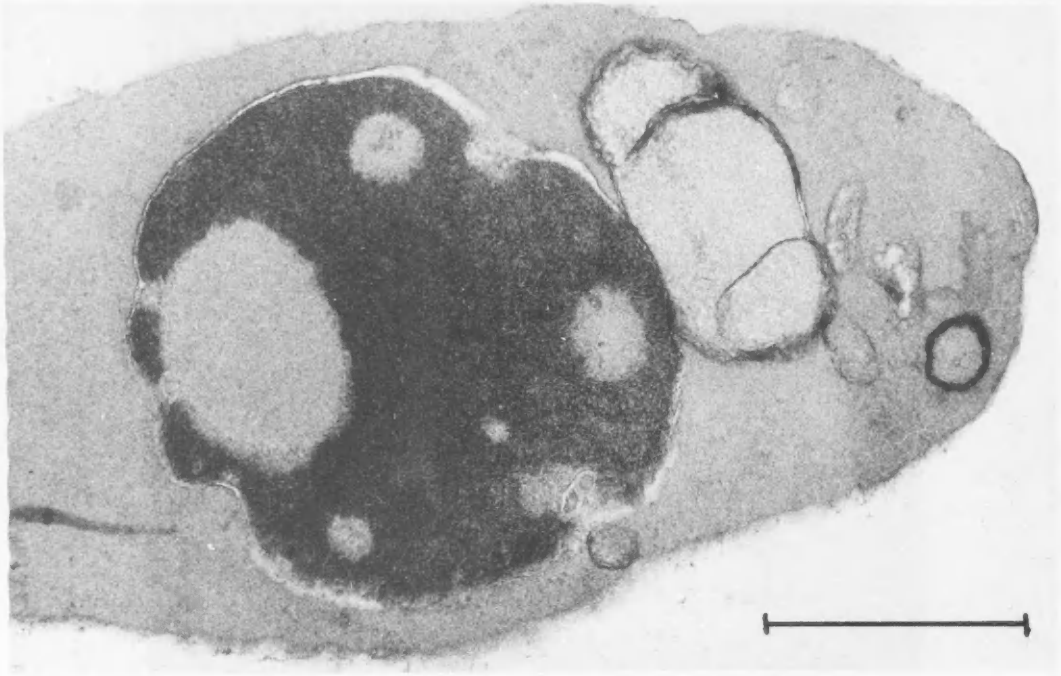


FIG. 5. Type II inclusion in erythrocyte with chromatin margination and light inclusion body surrounded by multiple membranes. Bar represents 1 μm.

Virions — These were hexagonal or pentagonal, 145 nm diam when measured edge to edge (mean of 55 particles from several preparations), and composed of an outer, apparently rigid, electron-dense layer 8 nm wide, a less electron-dense layer 16 nm wide, and a densely staining core approximately 100 nm diam, containing a 40-nm central electron-translucent area (Fig. 6).

In general the virions were distributed throughout the cytoplasm and not associated with a viroplasm. Frequently aggregates of virus were observed in the cells, arranged in a loose hexagonal array. In these instances there was no discernible spacing between adjacent particles, indicating the lack of an external electron-translucent envelope.

On partially purified preparations of PEN-infected herring erythrocytes, negative staining revealed hexagonal virions with a diameter of 195 nm and an outer and inner capsid, with no evidence of an external envelope (Fig. 7). The virions appeared similar to disrupted or partially degraded lymphocystis virions (Zwillenburg and Wolf 1968).

The herring PEN virions resemble those of cod and blenny viruses (Walker 1971; Appy et al. 1976; Johnston and Davies 1973) with some significant differences. First, the virus is smaller than the other two intraerythrocytic viruses of fish: cod PEN is 300–360 nm (Walker 1971; Appy et al. 1976), blenny PEN 200–300 nm (Johnston and Davies 1973), and herring PEN 145 nm. The presence of DNA and morphologic appearance of the herring virus support its inclusion in the Iridovirus group. Second, blenny (Johnston and Davies 1973) and cod viruses (Walker 1971; Appy et al. 1976) are surrounded by distinct, fibrillar electron-translucent region of the cytoplasm. At no time was a similar translucent area noted with the herring virus and the cytoplasm was always in apposition to the outer coat of the virion. The presence of virus aggregates whose capsids were in close contact makes the presence of an outer envelope on the herring PEN virions unlikely. Third, in contrast to the cod PEN virions (Walker 1971; Appy et al. 1976) the nucleoid contained a clearly discernible light core approximately 50 nm diam.

Thus, the intraerythrocytic virus of Atlantic herring appears to be a discrete entity, differing significantly from the other intraerythrocytic viruses of fish reported up to the present. A clarification of the relationships between the viruses of these marine fishes awaits the use of immunological and biochemical techniques.

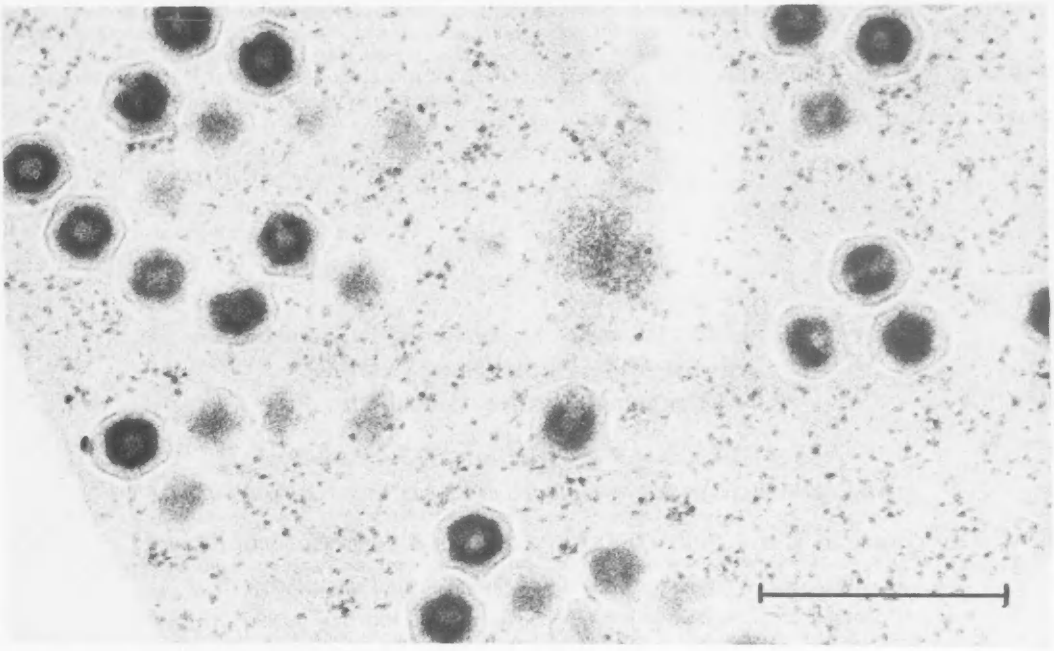


FIG. 6. High magnification electron micrograph of PEN virions showing the hexagonal capsid and doughnut-shaped nucleoid. Bar represents 100 nm.

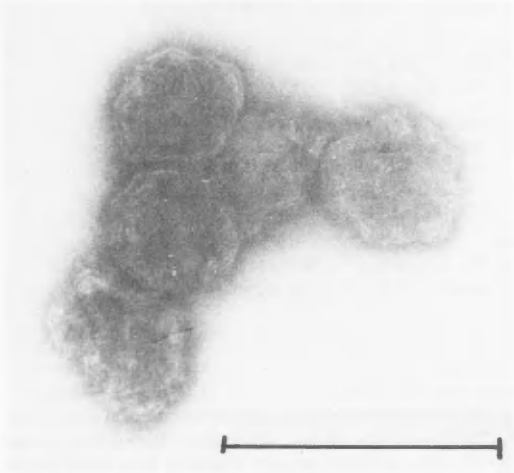


FIG. 7. Negatively stained preparation of partially purified herring PEN virions. Although virions appear damaged a clear double-layered capsid is apparent, and no envelope is evident. Bar represents 100 nm.

Acknowledgments — This investigation was supported by Public Health Service grant no. 1R01HL19164-01 from the National Heart and Lung Institute, National Institutes of Health, by grant no. PCM75-22747 from the National Science Foundation, and the Life Sciences and Agriculture Experiment Station of the University of Maine.

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Baeoctenus bicolor (Diptera: Chironomidae) Parasitic in Unionid Bivalve Molluscs, and Notes on Other Chironomid–Bivalve Associations

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GORDON, M. J., B. K. SWAN, AND C. G. PATERSON. 1978. *Baeoctenus bicolor* (Diptera: Chironomidae) parasitic in unionid bivalve molluscs, and notes on other chironomid–bivalve associations. J. Fish. Res. Board Can. 35: 154–157.

Parasitic association of *Baeoctenus bicolor* Sæther with the bivalves *Anodonta cataracta* and *A. implicata* in Morice Lake, New Brunswick, is described. An intimate relationship was also found between an undescribed genus of orthoclad (near *Phycoidella*) and *A. cataracta* but not *A. implicata*. Neither chironomid species was found in the other two genera of unionids that occur in the lake.

Key words: Baeoctenus bicolor, Chironomidae, parasitism, Unionidae, *Anodonta cataracta*

GORDON, M. J., B. K. SWAN, AND C. G. PATERSON. 1978. *Baeoctenus bicolor* (Diptera: Chironomidae) parasitic in unionid bivalve molluscs, and notes on other chironomid–bivalve associations. J. Fish. Res. Board Can. 35: 154–157.

Nous décrivons l'association parasitaire de *Baeoctenus bicolor* Sæther et des bivalves *Anodonta cataracta* et *A. implicata* dans le lac Morice, au Nouveau-Brunswick. Nous avons également découvert une relation intime entre un genre non décrit d'orthoclade (se rapprochant de *Phycoidella*) et *A. cataracta*, mais non *A. implicata*. Ni l'une ni l'autre espèce de chironomide n'a été trouvée dans les deux autres genres d'unionidés habitant le lac.

Received May 5, 1977
Accepted September 13, 1977

Reçu le 5 mai 1977
Accepté le 13 septembre 1977

THERE are numerous documented cases of a variety of chironomid larvae that live phoretically on mayfly and stonefly nymphs, blepharocerid larvae (Codreanu 1939; Steffan 1965), corydalid larvae (Hilsenhoff 1968), and two families of catfish (Friehofer and Neil 1967; Fittkau 1974). Other species are known to be parasitic on or in mayfly nymphs, sponges, bryozoans (Steffan 1965, 1968), and gastropods (Steffan 1965; Czeczuga and Bobiatyńska-Kśok 1968). Still other species occur in association with sponges and bryozoans (Steffan 1965, 1968), although the precise nature of this association is unknown. Beedham (1965, 1970, 1971) found rare instances of chironomid–bivalve mollusc associations in which the larvae of *Glyptotendipes* occurred in *Anodonta cygnea* and larvae of *Metriocnemus* in *Dreissena polymorpha*. However, both chironomid species are common benthic dwellers and were found in bivalves that had been experimentally damaged by the removal of sections of shell; the association may not occur in healthy bivalves.

During studies on the bivalves in Morice Lake, N.B., we observed numerous chironomid larvae within the mantle cavities of bivalves. In this note we present evidence that the larvae of one species are parasitic in two species of *Anodonta* for at least part of their life cycle.

Printed in Canada (J4814)
Imprimé au Canada (J4814)

TABLE 1. Occurrence of larvae and pupae (P) of *Baeoctenus bicolor* and larvae of "Genus near *Phycoidella*" in *Anodonta cataracta* from Morice Lake, N.B.

Date	No. bivalves	% infected	$\bar{x}$ infection	Range	Instars
		Baeoctenus bicolor			
1974					
May 21	10	70	1.40	0–5	IV (14)
June 3	10	70	1.80	0–8	III (1), IV (11), P (6)
June 25–Oct. 10	104	0	0	0	
1975					
May 9	20	20	0.35	0–2	III (7)
May 27	24	19	0.17	0–1	IV (4)
June 6	21	28.6	0.67	0–4	IV (9), P (5)
July 3	23	13	0.13	0–1	IV (2), P (1)
July 24–Nov. 3	209	0	0	0	
Nov. 22	25	8	0.08	0–1	III (2)
1976					
Jan. 27	20	5	0.05	0–1	III (1)
Apr. 20	52	11.5	0.13	0–2	III (4), IV (3)
May 4	53	7.5	0.09	0–5	IV (5)
May 26	42	26.2	0.38	0–4	III (2), IV (9), P (5)
June 15	48	4.2	0.04	0–1	IV (2)
		"Genus near *Phycoidella*"			
1975					
May 9	20	85	2.70	0–7	
May 27	24	45.8	1.25	0–12	
June 6	21	10	0.15	0–2	
July 3	23	21.7	0.30	0–2	
July 24	24	54.2	1.13	0–4	
Aug. 14	24	66.7	2.17	0–7	
Sept. 9	24	83.3	2.33	0–14	
Oct. 7	112	85.7	1.46	0–9	
Nov. 3	25	96	4.32	0–13	
Nov. 22	25	88	2.55	0–6	

while a further species shows an intimate, although not necessarily parasitic, association with one species of *Anodonta*.

Materials and methods — Morice Lake is a polymictic, mesotrophic reservoir located 3 km north of Sackville, N.B. (45°56′N, 64°21′W). Bivalves were collected in a basket drag towed behind a powered boat. In 1974 samples of 10–30 *A. cataracta* were collected at approximately 3-wk intervals from May 21 to early October. Sampling was expanded in 1975 to collect between 20 and 112 bivalves at 3-wk intervals between May 9 and November 22. In January 1976, 20 bivalves were removed from a holding cage placed in the lake prior to ice formation. From 42 to 53 bivalves were also collected at 3-wk intervals from April 20 to June 15, 1976.

The bivalves were measured for maximum length and opened over a glass dish by severing the adductor muscles. The tissues were examined for the presence of chironomids and their location and any obvious damage to the bivalve tissue were noted. The bivalve was then repeatedly washed with a jet of water which was collected in the dish along with any chironomids. The bivalves were sexed by examining wet mounts of gonadal tissue or by noting eggs or glochidia in the marsupia.

Chironomid larvae collected from the dish or directly from the bivalve were mounted and identified. Instars were separated on the basis of head capsule widths.

Representatives of the other three bivalve species found in Morice Lake were also examined by the method outlined; most extensive collecting was concentrated during the period when chironomids were found in *A. cataracta*.

Results — *Baeoctenus bicolor* larvae and pupae inhabit tubes constructed of particulate organic material, which are normally attached to the anteriodorsal surface of the gills near the labial palps of *A. cataracta*. Larvae do not display territoriality; as many as three larvae have been found attached to the gills on one side of the bivalve while the other pair of gills were free of infestation. That the larvae actively feed upon gill tissue is demonstrated by the large area of gill damage surrounding the site of attachment of each larval case. In situations of infestation by two or more larvae on the gills of one side of a bivalve, as much as 50% of the gill tissue is absent, presumably having been ingested. During the spring, bivalves that do not contain the chironomid do not display gill damage.

However, some of the bivalves collected shortly after the chironomid emergence period clearly display such damage. This becomes progressively less obvious in later collections until the damage is no longer easily detectable in bivalves collected in late October.

Substantial yearly fluctuations were observed in the frequency of occurrence, mean density, and period of occurrence of *B. bicolor* in *A. cataracta* from Morice Lake (Table 1). Larvae were first encountered in November, while pupae were first found in late May or early June. Hosts were free of *B. bicolor* from late June or early July until early November.

The other three species of bivalves found in Morice Lake are *Elliptio complanata* (Solander), *Lampsilis ochracea* (Say) and *Anodonta implicata* (Say). During the period of noninfestation of *B. bicolor* in *A. cataracta*, no chironomid larvae were found in these three species of bivalves. During the infestation period, 67 *E. complanata* and 36 *L. ochracea* were examined and were free of the chironomid. Because *A. implicata* is very rare in the lake, only six specimens could be obtained during this period, of which three were parasitized with between four and eight larvae.

An unidentified member of the Orthocladiinae, which is representative of a new genus (Sæther 1977), occurred with a frequency that suggests an intimate relationship with *A. cataracta*. This genus is near *Phycoidella*. Unfortunately, it was not possible to determine certain aspects of the life cycle of this species. The 2 yr in which extensive data were collected (1974, 1975) revealed similar seasonal patterns of frequency of infestation of the bivalves, with a substantial decrease in June, followed by a sharp rise throughout July and early August. The data for 1975 are given in Table 1. The percentage of *A. cataracta* harboring this chironomid reasonably parallels the average number of larvae per bivalve. However, in spite of these obvious seasonal differences, the 860 larvae examined all had a length of approximately 1 mm and a head capsule width of between 40 and 60 μm with no evidence of the presence of more than one instar. Pupae were not found and efforts to obtain adults by holding freshly collected groups of *A. cataracta* in covered aquaria at frequent intervals over the spring and summer were unsuccessful. These results suggest that the larvae leave the bivalve and pupate elsewhere, perhaps after passing through further larval instars outside the bivalve.

Because of their small size, the larvae were most frequently recovered through washing of the bivalve tissue. Larval cases were not observed and no obvious damage to the bivalves was noted. This genus was never found in the other three bivalve species.

During the course of the study occasional specimens of *Pseudochironomus*, *Phaenopsectra*, *Polypedilum* (2 spp.), *Dicrotendipes*, and *Micropsectra* were found in *A. cataracta* and *E. complanata*. However, these are common benthic species of chironomids and this occurrence in the bivalves is probably accidental.

Discussion — Larvae of *Baeoctenus bicolor* appear to invade *Anodonta cataracta* as instar III in winter and begin to actively feed upon gill tissue. Metamorphosis to instar IV is concentrated at about the time of the ice leaving the lake. Pupation is initiated in late May or early June and emergence is complete by late June or early July. Although nothing is known on the biology of the first two instars, the active feeding on bivalve tissue by the last two instars indicates that the association with the bivalve is a parasitic one during this phase of the life cycle. *Baeoctenus bicolor* is absent from *E. complanata* and *L. ochracea* but occurs in *A. implicata*. Sæther (1976) described *B. bicolor* from adults collected at Lake Winnipeg, where the only *Anodonta* is *A. grandis*.

The frequency of occurrence of larvae of the "Genus near *Phycoidella*" would also suggest an intimate association, although not necessarily a parasitic one, with *A. cataracta* although all larvae were representatives of one instar.

It is perhaps not unexpected that two species of chironomids are found to be either parasitic or show a close association with *A. cataracta* when the other rather common associations that have evolved between chironomids and other aquatic organisms are considered. The evolution of a parasitic association of *B. bicolor* with *A. cataracta*, *A. implicata*, and perhaps other *Anodonta* species, and the association of "Genus near *Phycoidella*" with *A. cataracta*, place the larvae in a habitat where there is abundant food either in the form of bivalve tissue or filtered seston, abundant oxygen as a result of the water filtration activities of the host, and where predation pressure is undoubtedly limited.

Acknowledgments — O. A. Sæther kindly identified the chironomids and supplied the information on *Anodonta grandis* in Lake Winnipeg. This study was supported by the National Research Council of Canada through Grant A-6299.

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Effects of Cage Culture on Growth, Abundance, and Survival of Resident Largemouth Bass (*Micropterus salmoides*)

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KILAMBI, R. V., J. C. ADAMS, AND W. A. WICKIZER. 1978. Effects of cage culture on growth, abundance, and survival of resident largemouth bass (*Micropterus salmoides*). J. Fish. Res. Board Can. 35: 157–160.

Growth, population size, and survival of resident largemouth bass (*Micropterus salmoides*) were estimated before, during, and after the cage culture of *Salmo gairdneri* and *Ictalurus punctatus*. Growth in length, length–weight relationship, and condition factor were similar among the periods; however, abundance and survival of largemouth bass increased through the 3 yr of investigation. Stomach content analyses showed that the bass fed on fishes (mostly *Lepomis macrochirus*), crayfish, insects, and zooplankton (predominantly entomostracans). Increase in the standing crops of *L. macrochirus* and entomostracans during the study periods have provided forage to the increased bass population and thus resulted in greater survival of the young and adult bass of the cage culture and postcage culture periods.

Key words: largemouth bass, *Micropterus salmoides*, cage culture, growth, abundance, survival

KILAMBI, R. V., J. C. ADAMS, AND W. A. WICKIZER. 1978. Effects of cage culture on growth, abundance, and survival of resident largemouth bass (*Micropterus salmoides*). J. Fish. Res. Board Can. 35: 157–160.

Les auteurs ont estimé la croissance, la taille de la population et la survie d'achigans à grande bouche (*Micropterus salmoides*) endémiques avant, pendant et après culture en cage de *Salmo gairdneri* et *Ictalurus punctatus*. La croissance en longueur, la relation longueur–poids et l'indice d'embonpoint sont identiques d'une période à l'autre; cependant, l'abondance et la survie des achigans à grande bouche augmentent durant ces 3 a qu'a duré l'expérience. L'analyse des contenus stomacaux démontre que l'achigan se nourrit de poissons (surtout *Lepomis macrochirus*), d'écrevisses, d'insectes et de zooplancton (en grande partie entomostracés). Une augmentation de la biomasse de *L. macrochirus* et d'entomostracés durant les périodes d'étude fournirent une nourriture suffisante à une population accrue d'achigans, entraînant ainsi une meilleure survie des jeunes achigans et des adultes durant la période de culture en cage et la période subséquente.

Received June 8, 1977
Accepted September 19, 1977

Reçu le 8 juin 1977
Accepté le 19 septembre 1977

Printed in Canada (J4872)
Imprimé au Canada (J4872)

TABLE 1. Back-calculated total lengths of largemouth bass.

Period	Weighted mean calculated total length (mm) at the end of year										
	1	2	3	4	5	6	7	8	9	10	11
Precage	98	200	261	295	332	365	398	431	453	491	480
Cage	98	201	259	310	338	361	365	391	—	—	—
Postcage	102	192	256	300	335	379	406	426	457	477	—
Combined	100	198	259	300	335	373	403	424	455	484	480
Increments	—	98	61	41	35	38	30	21	31	29	0

RECENTLY there has been growing interest in the culture of fish in cages in the United States. When feeding the caged fish, some of the food inevitably enters the surrounding water; fecal matter from caged fish is also added to the water. These may act as added nutrients and may bring about changes in the water quality and biota of a body of water.

Fishes respond to changes in the aquatic trophic systems directly or indirectly. Fishes experience higher growth rates following lake fertilization (Weatherly and Nicholls 1955; Munro 1961). Collins (1970) reported dense populations of bluegill (*Lepomis macrochirus*) and redear sunfish (*L. microlophus*) near culture cages. Loyacano and Smith (1975) stated that total number and weight of native fish captured at cage sites were significantly greater than at control sites during and after cage culture.

There have been no studies dealing with population size and growth of native fishes associated with cage culture. This note deals with the growth, abundance, and survival of largemouth bass (*Micropterus salmoides*) before, during, and after cage culture of rainbow trout (*Salmo gairdneri*) and channel catfish (*Ictalurus punctatus*) in Crystal Lake, Ark.

Materials and methods — Crystal Lake has a surface area of 24 ha, with average and maximum depths of 4.5 and 9.0 m, respectively. The investigation was divided into precage (November 1971–October 1972), during cage (November 1972–October 1973), and postcage (November 1973–October 1974) culture periods. During the cage culture period rainbow trout and channel catfish were fed 17,408 kg of feed for a gain of 8,673 kg in biomass (Kilambi et al. 1977).

Bimonthly samples of largemouth bass were collected by gill nets and electroshocker. Data on length and weight were recorded for each fish and a scale sample was taken from the tip of the left appressed pectoral fin. Distances from focus to each annulus and to the edge of the scale in the center of field were measured at a magnification of 40×. Age determinations were based on the number of scale annuli. Lengths attained at previous ages were calculated from the total length–scale radius relationship.

Largemouth bass populations were estimated by the Schumacher multiple census technique (Ricker 1975) at the end of the study periods. This method was chosen as it is not seriously affected if the marked fish are not randomly distributed. The fish were obtained by electroshocking during darkness and were marked by anal finclipping. Mark–recapture of largemouth bass was conducted for 6 days (September 20–October 3, 1972) for precage culture period, for 6 days (October 1–9, 1973) for cage culture period, and for 7 days (October 21–November 1, 1974) for postcage culture period.

Annual survival rates were estimated from the relative abundance of age-groups.

Results — Total length–scale radius relationship — A total of 377 largemouth bass were collected during the three study periods. Since the total length–scale radius relationship was not significantly different between sexes, the data for the sexes were pooled and the relationship was estimated as

$$L = 13.88 + 1.67S \quad (r = 0.92),$$

where L = total length (millimetres) and S = scale radius (millimetres).

Length–weight relationship — The relationship of weight to total length between sexes within the three study periods showed significant difference at the 0.01 level only during the cage culture period. Further analysis by the combination of probabilities test (Snedecor 1956) indicated no difference between sexes at the 0.05 level. Therefore, data for the sexes were pooled for each of the periods and covariance analysis showed no difference among the study periods. The length–weight relationship for all the periods is described by the equation

$$\log W = 3.09 \log L - 5.13141.$$

The coefficient of condition ($K = (W/L^3)\ 10^5$) was not different between the study periods at the 0.05 level; the Crystal Lake largemouth bass had an average K-value of 1.27.

Growth — The lengths of age-groups I through IV were compared between the periods. For each age-group differences in absolute growth were not significant between the periods at the 0.05 level. Further, the back-calculated lengths at different ages were similar between the study periods (Table 1).

Population estimates — The population sizes and their confidence intervals estimated by the Schumacher method are given in Table 2. From precage to cage cul-

TABLE 2. Largemouth bass population in Crystal Lake with 95% confidence ranges.

Period	Schumacher estimate	Confidence range
Precage	2573	2308–2909
During cage	3210	2274–5455
Postcage	4063	3232–5470

TABLE 3. Annual survival rates of the Crystal Lake largemouth bass.

Age interval	Precage culture	Cage culture	Postcage culture
0–1	—	—	0.27
1–2	0.47	—	0.37
2–3	0.50	0.55	0.55
3–4	0.50	0.55	0.64
4–5	0.50	0.61	0.70
5–6	0.50	0.52	0.82
6–7	0.50	—	0.82
7–8	—	—	0.86
Geometric mean	0.49	0.56	0.60

ture and cage culture to postcage culture periods, the largemouth bass population increased by 25 and 27%, respectively, with an overall increase of 58% from precage through postcage culture periods.

Survival rates — Annual survival rates (Table 3) increased through the cage culture and postcage culture periods, and survival of older age-groups increased throughout the study.

Discussion — Although largemouth bass population size and annual survival rate increased through the study periods, there was no change in growth, indicating sufficient availability of food organisms. Stomach content analysis by frequency of occurrence showed that in the precage culture period the bass fed on fishes (35%), crayfish (29%), insects (27%), and zooplankton (mainly entomostracans) (9%). The identifiable fishes in the diet were bluegill, green sunfish (*L. cyanellus*), and largemouth bass. During the cage culture period, insects (42%), fishes (28%), crayfish (18%), and zooplankton (12%) contributed to the diet; the fishes in the diet were lepomids (mostly bluegill), largemouth bass, crappie (*Pomoxis* sp.), and banded sculpin (*Cottus carolinae*). The post-cage culture bass fed mostly on fishes (46%), insects and crayfish (both 21%), and zooplankton (12%). The bluegill was the most abundant of the identifiable fishes in the diet, followed by redear sunfish, largemouth bass, and unidentifiable centrarchids.

Occurrence of zooplankton in the diet declined steadily after the bass reached 110 mm and was negligible in the diet of bass larger than 170 mm, fishes were consumed by all the length-groups of bass studied. Hodson (1966) reported that for bass <69 mm, entomostracans were important in the diet and for bass >100 mm, fish. For the Beaver and Bull Shoals Reservoir largemouth bass <40 mm, entomostracans were important part in the diet (Applegate and Mullan 1967). Olmsted (1974) reported that bluegill was the major forage fish for the Lake Fort Smith largemouth bass and the zooplankton in the diet was mostly composed of entomostracans.

Standing crop of bluegill of Crystal Lake increased by 134% by the end of the cage culture period, and in the postcage culture period it was 97% more than that of the precage culture period (Kilambi et al. 1976). Zooplankton standing crop increased during cage culture and subsequently dropped to near precage culture level during the postcage culture period; however, entomostracan populations increased throughout the study periods (Kilambi et al. 1976). Based on the food habits of the bass and the standing crops of bluegill and zooplankton, increased standing crops of bluegill and zooplankton apparently contributed to survival of young and adult bass of Crystal Lake; thus the increased bass population was not deprived of food. Hence the Crystal Lake largemouth bass of the three study periods had similar growth and condition factor.

Acknowledgments — We thank the Arkansas Game and Fish Commission and the U.S. National Marine Fisheries Service for providing funds through Public Law 88-309.

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BOOK REVIEWS/COMPTES RENDUS

Fishing with Light. *M. Ben-Yami. Fishing News Books Ltd., Surrey, England, 1976. 121 p.*

This little book reviews, in a condensed way, the present state of fishing with light throughout the world. Historical backgrounds of several modern practices are mentioned, for instance 15th century Dutch fishermen already using lights to attract herring, or fishing for squid with pitch pine burning in wire baskets as introduced by Chinese fishermen in California at the end of the 19th century. The author describes himself as a fisherman and fishing technologist. Accordingly, the emphasis of this manual is on practical aspects of its topic, i.e. types of light sources being used, in combination with specific types of boats and fishing tactics and techniques such as purse seining, electric and pump fishing, liftnetting, jigging. The bibliography contains 71 titles with a large portion from the USSR and Japan, reflecting the substantial research effort and success these two countries must be credited with in this field. Two chapters of the book deal, in a somewhat cursory manner, with facts underlying all light fishing techniques, namely physical properties of light in water, and response modes of fish towards artificial light. A reader who wants to move from the present largely historical–empirical stance through understanding of underlying mechanisms towards rational further development will not find sufficient information here. But then, it must be conceded that only the first of these two basic aspects, namely light in water, is well researched and documented, while current literature on responses to various light intensities and wavelengths under different ecological and physiological conditions does not lend itself to easy or safe deductions and generalizations. Nevertheless, this manual may well lead to a further expansion of light fishing techniques, simply by copying and empirically modifying some of the procedures described. The appended list of species being caught with light at present may prove useful here, e.g. by applying the highly successful Soviet technique for fishing kilka (*Clupeonella* sp.) to related species in other countries. The overall scarcity of data on freshwater species may indicate a potential waiting for development.

In summary, this booklet can be recommended to all fishermen and institutions looking for ways to improve fishing efficiency.

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(R134)

Grassy Narrows. *G. Hutchinson and D. Wallace. Van Nostrand Reinhold Ltd., Toronto, 1977. xiv + 178 p. $8.95.*

No Safe Place. *W. Troyer. Clarke, Irwin and Company Limited, Toronto and Vancouver, 1977. xvi + 267 p. $13.95.*

Both books are about the politics of pollution. The authors have taken a close look, don't like what they see, and are convinced that we must develop better ways of coping with the problems. Mercury contamination in the Wabigoon and English rivers is the example and a better example is hard to imagine. The prime ingredients are a major environmental contamination problem and people, industries, and governments that simply haven't been able to cope effectively with it. The authors see little evidence that we are learning from the lessons to be learned from examples like Dryden or Minimata.

The books, for the most part, are based on sound scientific and technical information but the message is much more than technical. Rather, the authors have marshaled the facts and gone beyond them to question and challenge the values of a society that appears to have neither the will nor the ability to cope with large scale environmental–social problems. Obviously the authors are motivated by a desire to help right the wrongs that have been perpetrated on those that have been forced to bear most of the human costs of this particular tragedy. Perhaps by documenting man's inhumanity to man in such graphic terms it will help us move to a more equitable and fair treatment of the affected communities and individuals like the Lamms and Mylses who showed a brand of courage that was clearly lacking in many of our government spokesmen and institutions. For many of course, it is too late — the murders, suicides, and the collapse of the social fabric of the communities have already taken a harsh toll. The clock cannot be turned back but there are some lessons to be learned.

In the authors' preface to *Grassy Narrows* they state that "It is our hope that the personal tragedies and political intrigues documented in these pages may move the reader to a deeper concern for the imminent danger we all face." Both books make it clear that what happens to Grassy Narrows, White Dog, the Lamms, and the countless others who are carrying the costs of other environmental tragedies that have occurred and are still occurring in Canada and around the world, should concern us and hopefully move us to constructive action. The reader who is seriously interested in understanding the ramifications of this particular environmental–social issue would do well to read both books. They are complementary. Both profess to have similar concerns and objectives but one has only to read to the table of contents to sense that they are very different in terms of focus, approach, scope, and outlook.

Grassy Narrows, for the most part, is a very moving documentary of some of the hardships, suffering, and deaths in a community that has been forced to carry a very unfair proportion of the environmental and social costs of an industry that offers them little in return. It's clear that the community simply is not coping. The symptoms and causes and likely results of the Grassy Narrows tragedy are treated in a manner that will provide useful insights, but the book gives little guidance as to how the concern that it could generate might be channeled into something constructive. In their words "the dilemma begged and defied solution." They were referring to the conflict over whether or not the contaminated waterway should be closed to sport fishing, but the statement could as well apply to the much broader questions that the book raises but doesn't really

attempt to answer. How do we help Grassy Narrows? Or in a more general sense how should our society distribute the environmental and social costs of industrial activities?

No Safe Place is a very different kind of book. It is also written with compassion and concern but the sense of resignation and helplessness that seems to permeate *Grassy Narrows* is usually replaced by other emotions — frustration, anger, and even outrage at the way officials, agencies, and governments have handled or mishandled the mercury crises in Northwest Ontario. Those who read the book will often be angered too, sometimes more by the author's approach than by the events. The actors in this drama are often treated very harshly, in some cases probably unfairly, and inevitably some will resent the way in which the author has marshaled the facts available to him. He identifies scores of villains who, because of ignorance or deliberate action or inaction, he believes have either contributed to the problem or failed to help resolve it.

The philosophy, analyses, and conclusions can be summed up by three quotations from the book. The following excerpt from the acknowledgments section seems to crystalize the author's philosophy and rationale for the book. "Our society has grown too complex for us to survive without the help of real experts — the people in government, business, industry, who know what's going on and are prepared to disclose that data. Our mutual responsibilities to avoid a permanently "silent spring" have far outstripped the need to avoid embarrassing a deputy minister, an executive vice-president or a shareholder."

In the last chapter appropriately entitled "No Safe Place" Troyer summarizes the lessons to be learned from the experience in Northwest Ontario. "Governments and their spokesmen and agencies lie when the truth is embarrassing; at the least they dissemble, delay, confuse and hide information. Industries also exaggerate or minimize when it's in their most pragmatic and intimate interests to hide error and duck culpability. Citizens unaware of the damage they may be suffering at the hands of the environmonsters will go on in ignorance if they depend exclusively on government/industry/regulatory agencies for help. Often these agencies fail through inadequate resources, political will, resolve and candour. None are likely to move far without the spur of informed, even angry, public constituency."

The last chapter ends with a brief conclusion section where he makes the following recommendations: "Some of the lessons of Northwest Ontario and the parallel examples we've examined are obvious: the crying need for major, publicly financed environmental/occupational health centres with the staffs, legislative muscle and resources to move, instantly and effectually in future cases. (There will be a case near where you live, the day you read this page — check your newspaper). We need real, enforced requirements that chemicals be proven safe before they are unleashed on us; we must have real, enforced requirement that polluters clean up after themselves, pay all of what it costs to remedy their assaults on our community." He then goes on to argue for more "sunshine laws" to guarantee freedom of information. In his words "Mushrooms thrive in the dark. Societies don't."

The text of the book is just as blunt and hard hitting as the quotations. It is well researched, in fact extremely well researched, and includes a great deal of information that could only have come from insiders upset enough with events to risk releasing confidential reports, studies, and internal memoranda. Undoubtedly, some officials will be angered at the release of this material and will disagree with the way it has been interpreted (some might say manipulated) to paint such a sinister picture of events and the motives of people who have shaped them. Aside from feeling a bit uneasy or defensive when the broadsides seemed too harsh or hit too close to home, I found his analysis of the interplay of environmental, social and institutional forces to be both comprehensive and astute. Certainly from my own experience I can recall many specific examples that are consistent with his analysis and conclusions although sometimes I would have been inclined to attribute them more to ignorance and accident rather than to deliberate, calculated actions.

The major shortcoming of the book in my opinion is that the concept of "publicly financed environmental/occupational health centres" was never really developed to where the reader could be sure what the author meant. After following and usually agreeing with his arguments concerning the "spasm response and crises management" syndromes of civil service bureaucracies, it was very difficult to see how the concept of publicly financed environmental occupational/health centers could actually be implemented within the Canadian context. Would it be too much to hope that Troyer and his team clarify, refine, and rationalize the concept in a manner that would be credible with the public, acceptable to their elected representatives, tolerated (even if begrudgingly) by the civil service bureaucracy, and at the same time create a research–information climate where scientists and medical researchers could do their job and be effective? With a sound concept and a clear rationale Troyer might be able to convince enough people that we can't afford to be without these safeguards.

No Safe Place will attract and hold the reader's attention and it could just become a very significant social–political document. The chances of this happening will be enhanced if the author, or perhaps someone else, refines and sells the message to the right audiences.

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Books Received / Livres reçus

August 1977

Adaptation to Environment. Essays on the Physiology of Marine Animals. *Edited by R. C. Newell. Butterworth and Co. (Publishers) Inc., Woburn, Mass., 1976. xiii + 539 p. $59.50.* (These essays review in detail the special adaptations of marine organisms to the particular environmental conditions that they are likely to encounter in their natural habitat. The book covers experimental work and conceptual frameworks.)

Fishexpo '76 Seminar. Report of Proceedings, Melbourne, 21–23 Sept. 1976. *Convened by H. E. Rogers. Australian Government Publishing Service, Canberra, Australia, 1977. xii + 762 p. $13.00 (Aust.).* (A massive compilation of the Proceedings of a seminar on "catching, processing and marketing of fish" from Australian waters.)

Fish Population Dynamics. *Edited by J. A. Gulland. John Wiley and Sons, Inc., New York, N.Y., 1977. ix + 372 p. $27.00.* (This book describes how the dynamics of fish populations can be analyzed in terms of the factors affecting their rates of growth, mortality, and reproduction, with particular emphasis on the effects of fishing.)

Handbook of Freshwater Fishery Biology. *Kenneth D. Carlander. Iowa State University Press, Ames, Iowa, 1977. Vol. 1. vii + 431 p. $18.00.* (This book serves as an index to the literature in fish biology with respect to the family Centrarchidae, including information on age, growth, length, weight, fecundity, reproductive behavior, feeding habits, and other characteristics. Ranges, habitats, and population data are also given.)

COMING NEXT MONTH/À PARAÎTRE LE MOIS PROCHAIN

Bias in using an age–length key to estimate age-frequency distributions
S. J. Westrheim and W. E. Ricker

Calculating mortality rates and optimum yields from length samples
G. G. Marten

Bioenergetics of pelagic fish: theoretical change in swimming speed and ration with body size
D. M. Ware

Stream habitats and behavioral interactions of underyearling and yearling Atlantic salmon (*Salmo salar*)
P. E. K. Symons and M. Heland

Phosphorus input and its consequences for phytoplankton standing crop and production in the Experimental Lakes Area and in similar lakes **D. W. Schindler, E. J. Fee, and T. Ruszczynski**

Deleterious effects of cyanide on early life stages of Atlantic salmon (*Salmo salar*)
G. Leduc

Direct potentiometric measurement of chloride in fisheries products by ion-selective electrode analysis
J. N. C. Whyte and J. R. Englar

Protein and lipid content of gonads, liver, and muscle of northern pike (*Esox lucius*) in relation to gonad growth **B. A. Medford and W. C. Mackay**

The price of knowledge: fisheries management as a research tool **W. Silvert**

A mathematical programming model for the management of a renewable resource system: the Kemano II development project **R. R. Everitt, N. C. Sonntag, M. L. Puterman, and P. Whalen**

A surplus yield model incorporating recruitment and applied to a stock of Atlantic mackerel (*Scomber scombrus*) **G. G. Walter**

Notes

Method for incubating leaf tissue to study decomposition in streams **D. C. Eidt and J. H. Meating**

Medusa predation and plankton dynamics in a temperate fjord, British Columbia
M. E. Huntley and L. A. Hobson

Survival of steelhead trout (*Salmo gairdneri*) eggs, embryos, and fry in air-supersaturated water
A. V. Nebeker, J. D. Andros, J. K. McCrady, and D. G. Stevens

Stability of freshwater sucker (*Catostomus* spp.) flesh during frozen storage
L. E. Dawson, K. L. Uebersax, and M. A. Uebersax

Feeding behavior repertoire of the alewife, *Alosa pseudoharengus*, and the ciscoes *Coregonus hoyi* and *C. artedii* **J. Janssen**

Journal of the Fisheries Research Board of Canada

Guide for Authors

Content

We welcome manuscripts reporting significant new information in the broad fields of fisheries management and technology, ocean science, and aquatic environments relevant to Canada. Manuscripts must not have been submitted or accepted for primary publication elsewhere. In general, manuscripts reporting fragments of research are unacceptable, as are those of mainly local interest.

Articles: Studies of broad scope that are original contributions to science.

Notes: Differ from articles on the basis of scope or completeness, rather than quality of research. They may report significant new knowledge arising from problems with narrow, readily defined limits, or important findings that warrant immediate publication before broader studies are complete. Notes should occupy no more than 3 printed (9 manuscript) pages.

Reviews: Detailed critical appraisals of broad areas of investigation, particularly those that have developed rapidly in recent years.

Perspectives: Essays of opinion or hypothesis on aquatic science that are of concern to the professional and layman. References are desirable; informal style is welcomed.

Letters and Comments: Opinion on topics recently discussed in the *Journal*; preferred maximum length, 500 words.

Editorial Practices

The *CBE Style Manual* (3rd ed. AIBS, Washington, D.C. 20016) is used as the authority in matters of form. The standards for spelling are: *Webster's Third New International Dictionary* and *le Grand Larousse Encyclopédique*. Titles of periodicals are abbreviated as in the *Biological Abstracts* list. References to literature are made by the name-and-year system. The *Journal* follows the names and spelling recommended in *A List of Common and Scientific Names of Fishes from the United States and Canada* (3rd ed. 1970) Spec. Publ. No. 6, American Fisheries Society. All measurements must be metric and SI units are preferred.

Series titles should be avoided. An *abstract* is required for all manuscripts, and should state concisely, in less than 250 words, what was done, found, and concluded. Up to 10 *key words* should follow the abstract.

Each manuscript is normally submitted to two *referees*. Contributors are encouraged to suggest names of potential referees.

Illustrations should be submitted in triplicate as photographic reproductions. They should not be mounted, stapled, or clipped. Symbols, letters, and numerals must be neat, and at least 1.5 mm high when the illustration is reduced to column width. Drawings should, when possible, be grouped to facilitate comparisons.

Proofs are sent to the author, who is responsible for correcting errors in typesetting. *Alterations to the content must be avoided.*

Fifty *reprints* are supplied free. Additional reprints may be purchased at the time of publication (an order form is sent with the proofs). A charge of $40.00 per page is made for articles published. When submitting a manuscript, an author should state that his institution will accept this charge.

Checklist on Manuscripts

Attention to the following questions will expedite appraisal of manuscripts by referees and editors.

Are the findings, interpretations, and conclusions adequately documented and relevant to the purpose of the study?

Are all the tables and figures needed, and organized to facilitate comparisons? Are there inconsistencies between tables or figures and the text, or within the text? Should some of the data be made available separately, in a data or manuscript report or at a data repository? If you use statistical analysis are the statistics subordinate to the research?

Would any of the text be clearer if condensed? Are summary statements given at the beginning of sections and paragraphs, and are details in sections and paragraphs relevant to their topics? Does the organization of the manuscript follow logically from the statement of purpose in the introduction?

Does the *title* encompass the content of the report? Does the *abstract* give the essentials of the new knowledge? Is the *introduction* largely limited to the scope, purpose, and rationale of the study? Is review of the literature limited to defining the problem? Are details of *materials and methods* limited to what scientists need in understanding the design of the study and in judging adequacy of the data? Are generalizations on the *results* adequate? Are findings distinguished from inferences from them? Is *discussion* limited to interpretation and significance of the findings?

Journal de l'office des recherches sur les pêcheries du Canada

Guide des Auteurs

Sujet

Les manuscrits comportant une information nouvelle et significative dans le vaste domaine de la gestion et de la technologie des pêches, des sciences de la mer et des milieux aquatiques au Canada sont les bienvenus. Ils ne devront pas avoir été soumis ou acceptés pour publication dans d'autres périodiques de fonds. En général, les manuscrits portant sur des fragments de recherche sont inacceptables, car ils n'ont qu'un intérêt local.

Articles: Etudes d'une portée générale, constituant des contributions inédites à la science.

Notes: Se distinguent des articles par la portée et le degré d'achèvement plutôt que par la qualité des recherches. Elles peuvent signaler une connaissance nouvelle et significative découlant de problèmes étroitement limités et facilement définis, ou d'importantes découvertes qui méritent d'être publiées avant que des études plus vastes ne soient terminées. Les notes ne doivent pas dépasser 3 pages imprimées (9 pages de manuscrit).

Critiques: Appréciations critiques détaillées de vastes champs de recherches, particulièrement dans les domaines ayant connu de rapides progrès au cours des dernières années.

Perspectives: Essais portant sur une opinion ou une hypothèse relative aux sciences aquatiques qui intéressent professionnels et profanes. Il est préférable de donner des références et d'écrire, si possible, dans un style simple.

Lettres et commentaires: Opinions sur des sujets récemment exposés dans le *Journal*. Longueur maximale préférable: 500 mots.

Disposition générales

Le *CBE Style Manual* (3e éd. AIBS, Washington, D.C. 20016) sert de guide quant à la forme de la présentation. Le *Webster's Third New International Dictionary* et le *Grand Larousse Encyclopédique* font autorité pour l'orthographe des mots. Les titres de périodiques sont abrégés suivant les *Biological Abstracts*. Les références bibliographiques dans le texte doivent suivre le système nom de l'auteur-année. Pour les noms de poissons et leur orthographe, le *Journal* suit les recommandations contenus dans *A List of Common and Scientific Names of Fishes from the United States and Canada* (3e éd. 1970) Publ. spéc. no 6, American Fisheries Society. Toutes les mesures doivent être conformes au système métrique, de préférence en unités SI.

On devra éviter les *articles en séries*. Un *résumé* doit accompagner chaque texte et doit exposer brièvement, en moins de 250 mots, ce qui a été fait, ce qui a été découvert et les conclusions auxquelles on est arrivé. Le résumé doit être suivi d'un nombre de *mots clés* ne dépassant pas 10.

Chaque texte est soumis d'ordinaire à deux *arbitres*. Nous encourageons les collaborateurs à suggérer les noms d'arbitres possibles.

Les *illustrations* doivent être soumises en triple sous forme de reproductions photographiques. Elles ne doivent pas être montées, brochées ni coupées. Les symboles, lettres ou chiffres doivent se détacher avec précision lorsque l'illustration est réduite à la largeur de la colonne. Les desseins doivent, autant que possible, être groupés pour faciliter l'étude comparative.

L'auteur doit revoir les *épreuves* qu'on lui remet et en corriger les erreurs typographiques. *Il doit éviter de changer le contenu du texte.*

Cinquante *tirés à part* sont fournis gratuitement. Des tirés à part supplémentaires peuvent être achetés au moment de la publication. (Un bon de commande à cet effet accompagne les épreuves.) Les frais sont de $40.00 par page pour la publication d'articles. Quand un auteur soumet un manuscrit, il doit préciser que son institution accepte les frais.

Liste de contrôle pour manuscrits

Une attention particulière aux questions suivantes facilitera l'évaluation des manuscrits par les arbitres et les éditeurs.

Les découvertes, interprétations et conclusions sont-elles bien documentées et pertinentes au but de l'étude?

Les tableaux et figures sont-ils tous nécessaires et agencés pour faciliter les comparaisons? Y a-t-il contradiction entre les tableaux ou les figures et le texte, ou dans le texte même? Est-ce que certaines données devraient être présentées séparément, dans un rapport manuscrit ou dans un dépôt de données? Si vous faites une analyse statistique, la statistique cède-t-elle le pas à la recherche?

Le texte y gagnerait-il en clarté s'il était plus condensé, en tout ou en partie? Contient-il des exposés sommaires au début de chaque section ou paragraphe, et les détails de ces sections et paragraphes sont-ils pertinents au sujet traité? La marche du manuscrit est-elle conforme à la logique énoncée dans l'introduction?

Le *titre* est-il représentatif du contenu du rapport? Le *résumé* donne-t-il l'essentiel des connaissances nouvelles? L'*introduction* se limite-t-elle à une esquisse de la portée, du but et des raisons de l'étude? La revue de la littérature se limite-t-elle à la définition du problème? La section *matériel et méthode* contient-elle le minimum de détails qui permettront aux autres scientifiques de saisir les grandes lignes de l'étude et de juger de la valeur des données? Les généralisations sur les *résultats* sont-elles adéquates? Permettent-elles de distinguer entre constatations et déductions? La *discussion* est-elle limitée à l'interprétation et à l'évaluation des découvertes?

Journal of the Fisheries Research Board of Canada

Guide for Typists Preparing Copy

Read "Guide for Authors" in the current volume of the *Journal*. When in doubt about how to type or arrange details, follow *Journal* practice.

Type the manuscript on white bond paper, not flimsy, preferably size 21.5 × 28 cm (8.5 × 11 inches). If possible, use paper with the lines numbered in the margin. Leave all margins at least 2.5 cm wide.

Double-space all parts of the manuscript, including tables, captions for illustrations, footnotes, and the reference list. Underline only material that is to be printed in italics. Indent the first line of each paragraph in the text and of each footnote.

In titles and headings, capitalize the first letter of each word. For small capitals, use small letters.

Indicate all text footnotes by superscript arabic numerals. Number them consecutively throughout the manuscript, starting with those on the title page. Place each footnote immediately below the reference to it, separating it from the text by horizontal lines.

Type the title, the authors' names and affiliations, related footnotes, the running head, the abstract with its bibliographic citation, and key words on the first page (see Appendix A). Number all pages beginning with the title page and including those for tables and captions for illustrations.

Have the author check the finished manuscript, and send the ribbon copy and two other clear copies to the Scientific Information and Publications Branch.

Abbreviations: Abbreviations used frequently in the *Journal* are listed in Appendix B.

Tables: Examine the current volume of the *Journal* to see how tables are set up (see Appendix C).
Note especially the following concerning *numbered tables*:

Type each on a separate page, using paper without the lines numbered.

Number them with arabic numerals.

Use horizontal lines above and below the headings and below the columns, and seldom elsewhere. Never use vertical lines; leave extra spaces instead.

Indicate table footnotes by superscript small letters and type them below the table.

Place the tables after the list of references.

Where each table is first referred to, type in the margin "Table — near here."

Note that *text tables* are not numbered, are typed with the text, and seldom need horizontal lines (see Appendix C).

Illustrations: Send three photographic reproductions (no larger than 21.5 × 28 cm) of each illustration with the manuscript. Type the author's name and figure number on a strip of paper and fasten it to the back of the print. Do not write or type on the illustration itself. Also, type the captions in succession on a separate page.

Where each figure is first referred to, type in the margin "Fig. — near here."

Mathematical Expressions, Equations, and Formulas: Leave three spaces above and below lines that have much material in superscripts, and above and below equations and formulas. In the various expressions, place each component in its correct position (see Appendix D).

Show all symbols clearly. In the margin, spell out Greek letters the first time they occur, and distinguish between the small letter *l* and the numeral *one*, and between the capital *O* and the numeral *zero*. Make other explanatory notes similarly.

References: Check the text to see that all references in the list are referred to. Type the reference list, double-spaced, in the style used in the *Journal* (see Appendix E). Type names of journals in full.

Headings: If the levels of importance of your heads and subheads are not apparent from their positions, identify each of them with an appropriate circled number or letter in the left-hand margin of the typed page: 1 or A for the highest level, 2 or B for the second level, 3 or C for the third level. Or include with your manuscript a typed list of heads and subheads, indented as necessary to indicate their relative importance.

Journal de l'office des recherches sur les pêcheries du Canada

Guide pour les secrétaires d'auteurs

Lisez le « Guide des auteurs » reproduit dans l'édition courante du *Journal*. Si vous avez des doutes sur la façon de dactylographier le texte ou d'en agencer les détails, suivez la pratique du *Journal*.

Dactylographiez le manuscrit sur papier blanc de bonne qualité, et non sur papier pelure, mesurant de préférence 21.5 × 28 cm (8.5 × 11 pouces). Si possible, employez un papier avec lignes numérotées en marge. Laissez toujours une marge d'au moins 2.5 cm.

Tout le manuscrit, y compris tableaux, légendes de figures, renvois et références bibliographiques, devra être dactylographié à double interligne. Ne soulignez que les mots qui devront être imprimés en italique. Mettez en retrait la première ligne de chaque paragraphe du texte et de chaque renvoi.

Dans les titres et les en-têtes, n'employez la majuscule que pour la première lettre de chaque mot. Pour les petites majuscules, employez les minuscules du dactylographe.

Indiquez tous les renvois du texte par des chiffres arabes suscrits. Numérotez les renvois consécutivement par tout le texte, à commencer par ceux de la page titre. Placez chaque renvoi directement sous sa référence, le séparant du texte par des lignes horizontales.

Sur la première page, dactylographiez le titre, les noms et affiliations des auteurs, les renvois qui s'y rapportent, le titre courant, le résumé avec sa référence bibliographique et les mots clés (voir annexe A). Numérotez toutes les pages, à commencer par la page-titre, de même que celles contenant les tableaux et les légendes des illustrations.

Voyez à ce que l'auteur vérifie le manuscrit final, et soumettez la première copie ainsi que deux copies lisibles à la Direction de l'information et des publications scientifiques.

Abréviations: Nous donnons à l'annexe B une liste d'abréviations communément utilisées dans le *Journal*.

Tableaux: Consultez une copie du *Journal* pour la composition des tableaux (voir annexe C).

Portez une attention particulière à ce qui suit au sujet des *tableaux numérotés*:

Dactylographiez chaque tableau sur feuille séparée, vous servant de papier sans lignes numérotées.

Numérotez les tableaux en chiffres arabes.

Tracez des lignes horizontales au-dessus et au-dessous des entêtes et au-dessous des colonnes, rarement ailleurs. Ne tracez jamais de lignes verticales; laissez plutôt des espaces supplémentaires.

Indiquez les renvois dans les tableaux par les minuscules suscrites et placez les renvois au bas du tableau.

Placez les tableaux à la suite des références bibliographiques.

A l'endroit où le tableau est mentionné pour la première fois, indiquez dans la marge « Tableau — près d'ici ».

Remarquez que les *tableaux dans le texte* ne sont pas numérotés, sont dactylographiés avec le texte et ne requièrent que rarement des lignes horizontales (voir annexe C).

Illustrations: Envoyez avec le manuscrit trois reproductions photographiques (21.5 × 28 cm ou moins) pour chaque illustration. Dactylographiez le nom de l'auteur et le numéro de la figure sur une lisière de papier que vous collerez au dos. N'écrivez pas à la main ou à la machine sur la photographie même. De plus, dactylographiez les légendes, dans l'ordre, sur feuille séparée.

A l'endroit où chaque figure est mentionnée pour la première fois, inscrivez en marge « Fig. — près d'ici ».

Equations, formules et expressions mathématiques: Laissez trois espaces au-dessus et au-dessous des lignes renfermant des chiffres ou des lettres suscrits et souscrits, ou encore au-dessus et au-dessous d'équations et de formules. Placez chaque composante des différentes expressions dans la position qui lui est propre (voir annexe D).

Indiquez clairement tous les symboles. Epelez en marge les lettres grecques, la première fois qu'elles apparaissent. Faites une distinction entre la lettre *l* et le chiffre *un*, et entre la majuscule *O* et la chiffre *zéro*. Insérez de la même façon toute autre note explicative.

Références: Vérifiez le texte afin de vous assurer que toutes les références apparaissant dans la liste bibliographique sont bien mentionnées dans le texte. Dactylographiez à double interligne dans le style en usage dans le *Journal* (voir annexe E). Ecrivez les noms des périodiques au long.

En-têtes: Si la position de vos en-têtes et sous-en-têtes ne suffit pas à indiquer leurs niveaux d'importance, identifiez chacun d'eux par un chiffre ou une lettre encerclé à la marge gauche de la page dactylographiée: 1 ou A pour le niveau supérieur, 2 ou B pour le deuxième niveau, 3 ou C pour le troisième niveau. Ou encore, transmettez avec votre manuscrit une liste dactylographiée d'en-têtes et de sous-en-têtes rentrés de façon à indiquer leur importance relative.

Announcements/Avis

Index

An author–subject index and list of all publications issued during 1977 will be supplied in early 1978, and distributed free to all *Journal* recipients, including subscribers. Additional copies will be available through the Scientific Information and Publications Branch.

Index

Un index par sujet et auteur et une liste de toutes les publications parues en 1977 paraîtront au début de 1978 et seront distribués gratuitement à tous ceux qui reçoivent le *Journal*, ainsi qu'aux abonnés. On pourra se procurer des exemplaires supplémentaires à la Direction de l'information et des publications scientifiques.

Subscriptions

Subscription rates effective January 1978 are $40 per year in Canada and $48 per year in other countries. Single issues are $4 and $4.80, respectively.

Abonnement

À compter de janvier 1978 les frais d'abonnement annuel seront de $40 pour le Canada et de $48 pour les autres pays. Chaque numéro coûtera $4 au Canada et $4.80 à l'étranger.

Call for Papers

The 1978 annual meeting of the American Fisheries Society will be held August 21–25 at the University of Rhode Island in Kingston. Organizers/conveners for symposia, modules, debates, and poster sessions are needed. Priority will be given to innovative and/or provocative topics. Those interested in organizing such a session should contact the program chairman with a tentative topic prior to January 31, 1978. The deadline for abstracts for contributed papers is March 10, 1978.

Dr Richard W. Gregory
Program Chairman 1978
Cooperative Fishery Research Unit
Lewis Hall, Montana State University
Bozeman, Montana 59717, USA

Appel de communications

La prochaine assemblée annuelle de l'American Fisheries Society se tiendra à l'Université Rhode Island, à Kingston, du 21 au 25 août 1978. Nous sommes à la recherche de responsables qui se chargeraient de l'organisation et des invitations pour les symposiums, les modules, les débats et les expositions d'affiches. Nous accorderons la préférence aux sujets innovateurs ou stimulants. Les personnes intéressées sont priées de communiquer avec le président du programme pour lui soumettre un texte préliminaire avant le 31 janvier 1978. La date limite pour la remise des résumés des communications est fixée au 10 mars 1978.

M. Richard W. Gregory
Président du programme, 1978
Cooperative Fishery Research Unit
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Bozeman, Montana 59717 É.-U.

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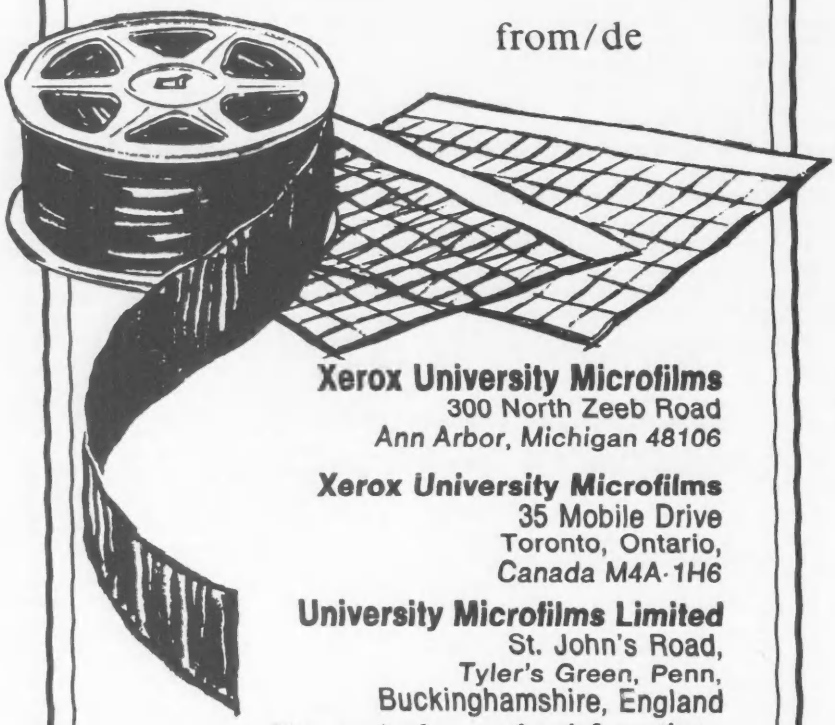

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Ultrastructural studies of piscine erythrocytic necrosis (PEN) in Atlantic herring (*Clupea harengus harengus*)
Paul W. Reno, Marine Philippon-Fried, Bruce L. Nicholson, and Stuart W. Sherburne 148–154

Baeoctenus bicolor (Diptera: Chironomidae) parasitic in unionid bivalve molluscs, and notes and other chironomid–bivalve associations
M. J. Gordon, B. K. Swan, and C. G. Paterson 154–157

Effects of cage culture on growth, abundance, and survival of resident largemouth bass (*Micropterus salmoides*)
Raj V. Kilambi, James C. Adams, and William A. Wickizer 157–160

Book Reviews/Comptes rendus: Fishing with light (M. Ben-Yami) **E. Scherer**; Grassy Narrows (G. Hutchinson and D. Wallace) and No safe place (W. Troyer)
A. L. Hamilton 161–162

Books Received/Livres reçus 163
Coming next month/À paraître le mois prochain 164
Guides for authors and typists/Guide des auteurs et Guide pour les secrétaires d'auteurs *i–iv*
Announcements/Avis *v*

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Journal of the Fisheries Research Board of Canada

Journal de l'office des recherches sur les pêcheries du Canada

JFRBAK35(1)1–164(1978)
ISSN 0015-296X

Volume 35, No. 1, January 1978

Volume 35, nº 1, janvier 1978

Relation of water temperature to infections of coho salmon (*Oncorhynchus kisutch*), chinook salmon (*O. tshawytscha*), and steelhead trout (*Salmo gairdneri*) with *Aeromonas salmonicida* and *A. hydrophila*
W. J. Groberg Jr., R. H. McCoy, K. S. Pilcher, and J. L. Fryer 1–7

Relation of water temperature to bacterial kidney disease in coho salmon (*Oncorhynchus kisutch*), sockeye salmon (*O. nerka*), and steelhead trout (*Salmo gairdneri*)
J. E. Sanders, K. S. Pilcher, and J. L. Fryer 8–11

Feeding interrelations between age-groups of pumpkinseed (*Lepomis gibbosus*) and comparisons with bluegill (*L. macrochirus*) **Allen Keast** 12–27

Enhancement of autotrophic production by nutrient addition in a coastal rainforest stream on Vancouver Island **John G. Stockner and K. R. S. Shortreed** 28–34

Toxicity of cadmium to goldfish, *Carassius auratus*, in hard and soft water
L. S. McCarty, J. A. C. Henry, and A. H. Houston 35–42

Clinal variation in scale characters of Atlantic salmon (*Salmo salar*) based on discriminant function analysis **W. H. Lear and R. K. Misra** 43–47

Movements of stream-resident and anadromous Arctic char (*Salvelinus alpinus*) in a perennial spring on the Canning River, Alaska **P. C. Craig** 48–52

Fish population structure in arctic lakes **G. Power** 53–59

Analysis of growth of Atlantic salmon (*Salmo salar*) in a small Newfoundland river
E. M. P. Chadwick, T. R. Porter, and P. Downton 60–68

Relation between temperature and incubation time for eggs of chinook salmon (*Oncorhynchus tshawytscha*) **D. F. Alderdice and F. P. J. Velsen** 69–75

Seaward migration of hatchery-reared Atlantic salmon, *Salmo salar*, smolts in the Penobscot River estuary, Maine: riverine movements
Stephen M. Fried, James D. McCleave, and George W. LaBar 76–87

Dechlorination of water for fish culture: comparison of the activated carbon, sulfite reduction, and photochemical methods **Gregory L. Seegert and Arthur S. Brooks** 88–92

Seawater adaptation and parr–smolt transformation of juvenile Atlantic salmon, *Salmo salar* **G. J. Farmer, J. A. Ritter, and D. Ashfield** 93–100

Distribution and morphology of the rodlet cell in fish
C. M. Morrison and P. H. Odense 101–116

Geographic variation in Atlantic cod, *Gadus morhua*, off eastern North America: a biochemical systematics approach **T. F. Cross and R. H. Payne** 117–123

Utility of meristic and morphometric characters for identification of capelin (*Mallotus villosus*) stocks in Canadian Atlantic waters
Joan C. Sharp, Kenneth W. Able, William C. Leggett, and James E. Carscadden 124–130

Notes

Reproductive isolation between pumpkinseed (*Lepomis gibbosus*) and longear sunfish (*L. megalotis*) (Centrarchidae) in the Thames River, southwestern Ontario
Miles H. A. Keenleyside 131–135

Estimating the diet of a sluggish predator from field observations
Charles H. Peterson and Brian P. Bradley 136–141

Unusual response of a cupric ion electrode in prairie lake waters **J. Barica** 141–143

Technique for tagging small fish with I^{131} for evaluation on predator–prey relationships
Gerard J. FitzGerald and Miles H. A. Keenleyside 143–145

Variation in age at first spawning in Atlantic salmon (*Salmo salar*)
Gunnar Nævdal, Marianne Holm, Oscar Ingebrigtsen, and Dag Møller 145–147

(Continued inside back)